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- Neuroscience (miscellaneous)

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How to publish in this journal

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Scope

An international multidisciplinary journal devoted to fundamental research in the brain sciences. Brain Research publishes papers reporting interdisciplinary investigations of nervous system structure and function that are of general interest to the international community of neuroscientists. As is evident from the journals name, its scope is broad, ranging from cellular and molecular studies through systems neuroscience, cognition and disease. Invited reviews are also published; suggestions for and inquiries about potential reviews are welcomed. With the appearance of the final issue of the 2011 subscription, Vol. 67/1-2 (24 June 2011), Brain Research Reviews has ceased publication as a distinct journal separate from Brain Research. Review articles accepted for Brain Research are now published in that journal.

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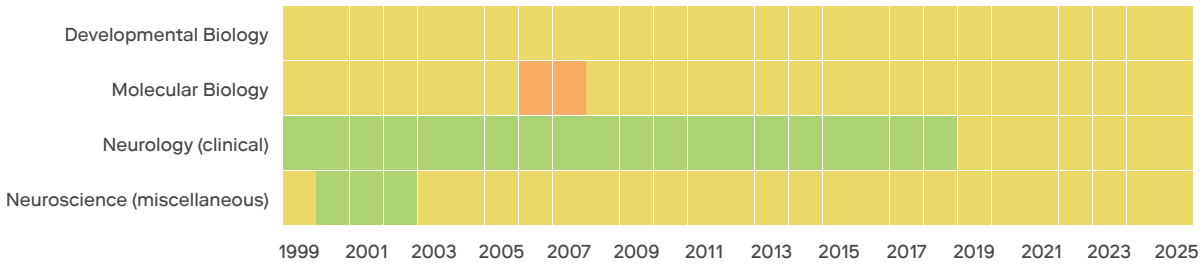

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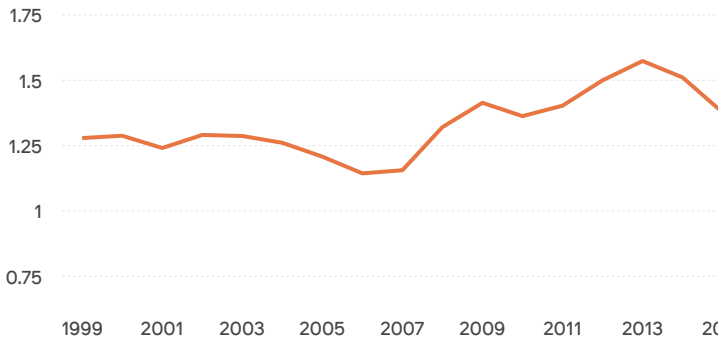

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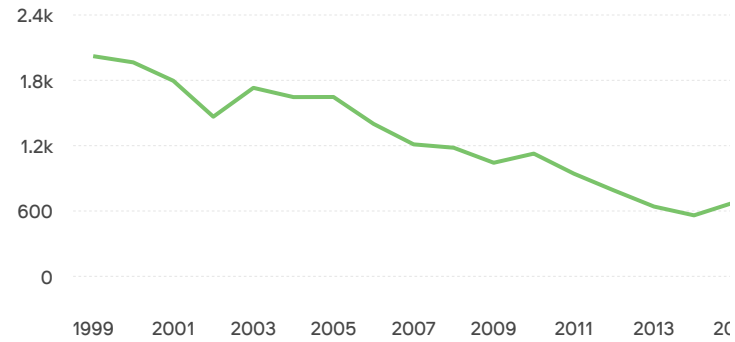

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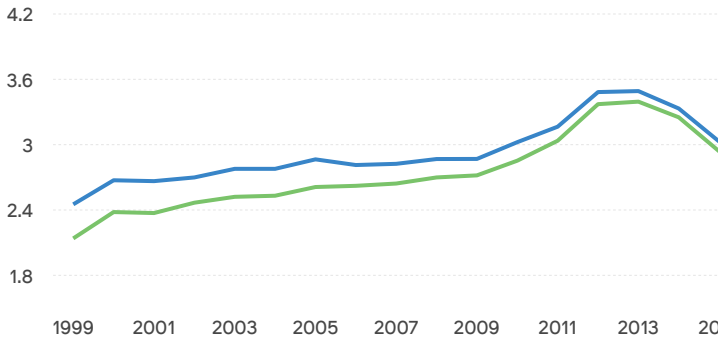

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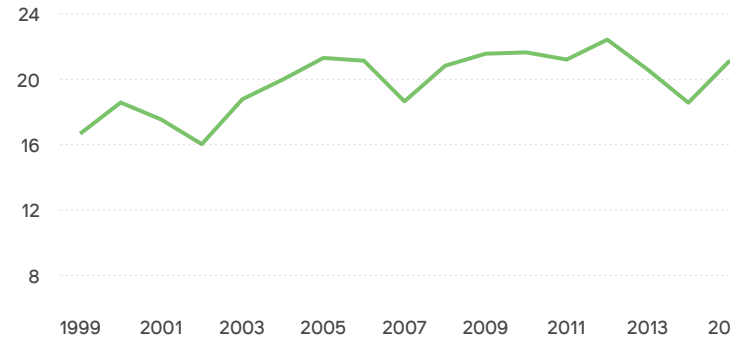

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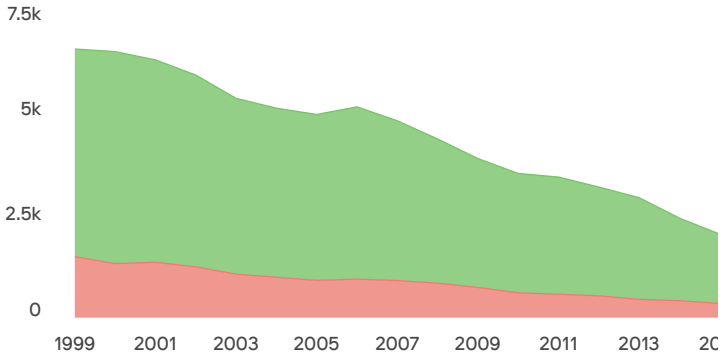

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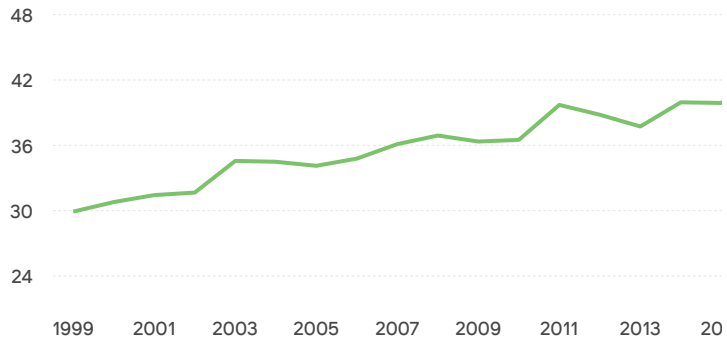

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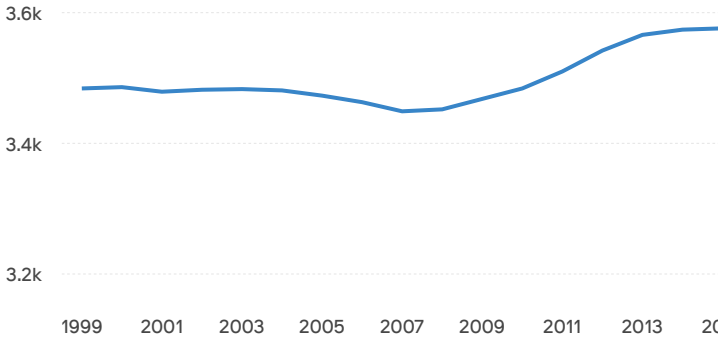

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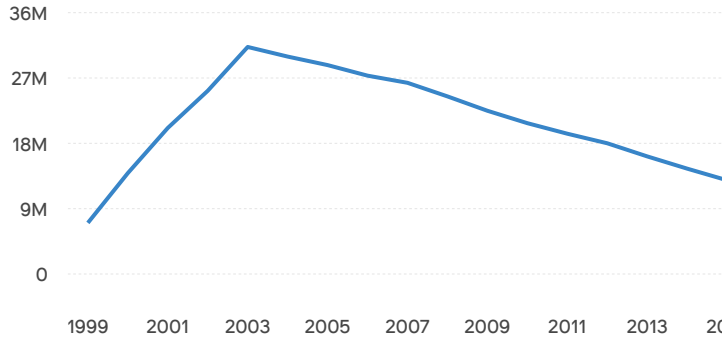

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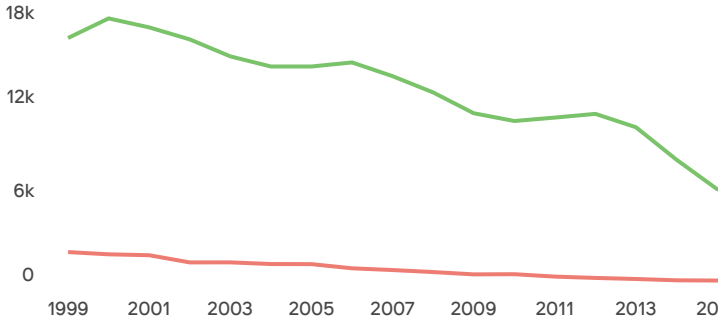

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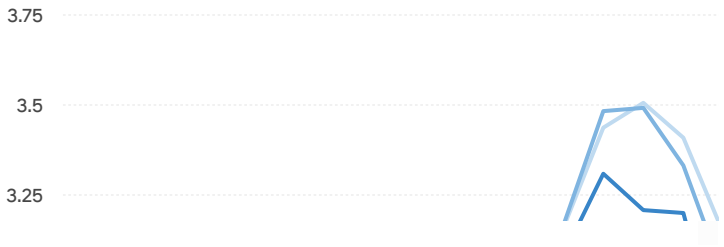

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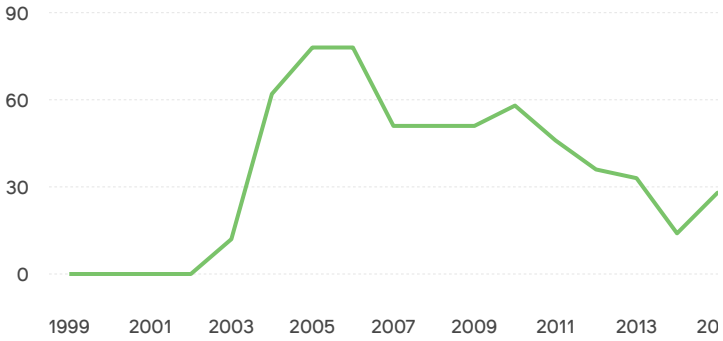

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P **paolo manzelli**
7 years ago

by Paolo Manzelli. (egocreanet2016@gmai.com)
A new parading for the implementation of the healthy function of the

Opening lecture to: Florence Laser 2019 , << 07/09 NOV. 2019 - Florence >>.
see: http://www.longolaser.it/convegni

Key concepts: BIOINFORMATION; LASER THERAPY AND BIOPHOTONS EMISSION. NEW APPROACH OVER THE STATE OF THE ART TO POWER THE COHERENT HEALTH STATE THROUGH LASER STYMULATION OF INTERCELLULAR COMMUNICATION
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“There is a crack in everything, that's how the light gets in”: Leonard Cohen's “Anthem”
https://www.youtube.com/watch?v=mX2xlW7Oa9c

Premise:
It is known that in light of the Ultra violet (UV. A), Visible and Near Infrared (NIR) spectrum it helps to heal wounds, injuries and burns; in fact, for over 50 years Medicine has made significant improvements in "Laser Light Therapies and also with Visible Light (LEDs)", which show significant progress in the treatment of a wide variety of medical treatments.

We are in fact aware that the "Light Therapies" induce an increase in the production of ATP, exciting the activity of the Mitochondria which can also act on the regulation of oxidative stress, and promote immuno-modulation, with the result of relieving pain associated with arthrosis and other forms of medical treatment that are positively influenced by phototherapy, through the use of specific light intensities and frequencies (between 200 nm and 1000 nm). These frequencies act to improve the synergistic coherence of the activity Redox of oxygen metabolism. We must note that as the successes of the "Laser // Led" medicine multiply at the same time, the complexity of specific methodological protocols for the application of various cell bio-modulation symptoms with light increases. As a result, the complexity of the treatment of pathological symptoms converges in highlighting that there is still a really clear "general theory of photo-biology", able to understand the causes of the treatment of different pathologies in order to avoid the skepticism that still pervades the full credibility and understanding of Light-Therapy.

Therefore following my speech at the conference on Florence Laser 2017, I this year

2019, I will try to place the developments of the Laser // Led medicine in a broader context of the paradigm shift, which sees the Care of Light in the dimension of the interactions between Epigenesis and Genetics that are expressed in terms of "quantum biomodulation" of the interactions between Energy, Matter, and Information.

Target:
The intent of my intervention is aimed at overcoming the lack of a theoretical understanding of the action of Laser and LED light, on various aspects of established medical care, in order to avoid the permanence of a wide disbelief that still remains as the main deterrent of scientific reliability of the treatment obtained through the photo-bio-modulation of the human body and brain.

The Search for a New Paradigm: "Outside of the Thinking Box"
The new paradigm essentially concerns a new way of thinking, related to a renewed vision of cognitive reference, such that it allows to put in a new order the obsolete ways of scientific thought
As a "mental experiment" let us now see how the interpretation of the photo-bio-modulation action of light can change, when the reference framework of scientific observation that is normally included in the traditional mechanical-thermodynamic approach is radically modified.

If indeed. I now argue that "we are made of water and light" (as the bio-physicist Fritz-Albert Popp asserts), it is easy to expect to receive demonstrations of disbelief, scornicism and inattention from those who are still convinced that we are mainly composed of protein, fat and sugar molecules and also that, due to food metabolism, we only emit heat instead of energy in the form of weak light-quantum sparks. (2)

Despite the incredulity and mistrust of the previous assertion, we can easily see that the average composition of the adult man is actually 65% water, and that the brain contains the highest concentration (about 80%), and that finally, rather than heat (IR and Microwave) we spontaneously emit a spectrum of Light, which transmits biological information in the range between Ultraviolet (UVA), Visible and near Infrared (NIR). (3)

The Paradigm Shift
This substantial change in Vision, precisely because it is verifiable, allows us to re-modulate obsolete conceptions of the effective functionality of DNA genetic expression, depending on the interaction with the Laser or Led Light.

In fact it is understood that the two main DNA (Nuclear and Mitocontrial) are able to function as piezoelectric sensors of a semi-crystalline nature, which interact by resonance (or by other quantum interactions such as fluorescence) with light, modifying electromechanical transduction of light signals in the articulation of the necessary movements and make the genetic expression highly specific. In particular with the Laser Therapy, one denotes eg. that the DNA of mitochondria acts as a photo-receptor as it allows the modulation of the production of Biological energy (ATP // ADP), and furthermore favors the coordination of the time of programming of apoptosis in order to favor cell renewal.

The studies of prof. Popp and other scientists have shown that the two DNAs spontaneously emit a high percentage of bio-photons (= biological light quanta). Therefore from the deepening of these frontier knowledge, it becomes understandable how the light Lases and Led can interact bio-quantically, exciting or modifying the internal communication of bio-photons, which presides over the synergistic coherence of the biochemical interactions that act in improving health and psycho-physical well-being. (4)
This new approach to the paradigm of life sciences, both medical and biological, modifies the pharmacological tendency, still today the majority, which is that of attributing the function of annealing of living matter to the "local" properties of single genes, as an action obtainable by contact (DNA / RNA) and of interchange of the properties of single molecules.

Differently admitting the prevalence of the "functionality of water and light" in determining the coherence of the biological coordination of living systems, today the new theoretical conception on the functioning of DNA, as a receptive-transmitting antenna of bioquantical signals, proposes a more "" enlightened "explanation of the obvious success in various fields of medicine based on the photo-bio-modulation of Laser or LED light. In fact, the emerging new paradigm of Bioquantic science tends to shift scientific attention to communication activity" at a distance "of the non-local interaction vibrational-electromagnetic properties" which make the integration between Epigenetic and Genetic information easier to understand.

The essential tool of such remote interaction lies in the function of "Rice Antenna-DNA transmitter", exercised through the emission of quantum electromagnetic signals (bio-photons and bio-phonons) which is based on considering DNA as a semi-piezoelectric crystal that translates the electromagnetic oscillations of light signals into specific movements of the double helix organized in such a way as to coordinate the genetic expression with the epigenetic information assumed by the environment. . (5)

- Light modulation of circular synchronism of both modalities if Synaptic Gaps
There are two main modalities of synaptic transmission: chemical and electrical. evidence indicates that these two modalities of synaptic transmission of energy and information closely interact, both during the brain -body communication exchanges. New trends in Neurotransmission tell us that the exchange of communication is realized between electric synapses which togheter form a cycle of transmission of energy and infoemation

The neuronal system is composed of neurons that contain a high percentage of water and glial cells (from Glia = glue) which are 5 times higher in number than the neurons. For a long time it was considered that the glial cells, (among them the astrocytes and the oligodendrocytes etc.), being composed mainly of fats (triglycerides) served only as energy support to the neurons. The Biochemical synapses that connect neurons are more visible to the NMR-Images technologies than the Bio-Electric ones that were discovered by Santiago Ramon Y Cajal and by Camillo Golgi (nobel in medicine in 1906) for the different absorption of an discovered dye from Golgi. Bioelectrical synapses are controlled by glial cells that supply energy to activate light sparkles through the gap of the crack where the flow of calcium ions (Ca ionic current) becomes asymmetric.

The biochemical synapses metabolize in communicating the neuro transmitters (Acetylcholine, Noroadrenaline, Dopamine, Adrenaline, Adenosine and other short chain of the upper hemispheres) each with exciting or calming functions with which the sequence of neuronal impulses is translated into biological responses. Otherwise, Bioelectric Synapses use the transformation of White Fat into Brown Fat, to use most of the Oxygen that is consumed in the brain (20% of the total) in order to produce energy in the form of "spark of light" in the gap of bioelectric fissures. These spark of light therefore have the function of activating / deactivating the entire "mind-body" information cycle by acting as ON / OFF switches to catalyze or deactivate the communication of neuronal impulses that run in the axons.
The electric synapses surround the single neurons and their Sparks of light also serve to position and sometimes reposition the neurons in the brain structure in 3 Dimensions, decreeing the functional specialization of each single neuron.

Therefore, overall, the "Glie-Neuron Network" cyclically controls Mind-Body neuronal communication. This circularity between the action of bio-electric and bio-chemical synapses constitutes a new trend of understanding of the Neuro-Sciences, based on the Glio-transmission of the flow of calcium ions, precisely because it cyclically controls the modulation of neuronal activity of the biochemical synapses.

The cyclical action between bio-chemical bio-electric synapses overcomes the linear conception that still admits that brain function is substantially generated by the bi-directional transfer of information between neurons and that deriving from the five senses of the body neglecting the complementary circular action to bio-electric synapses.(6)

The Fourth state od Water

Finally it is important to analyze the role of "intra-cellular water communication network" bearing in mind that surface water (called Fourth State, that is, EZ-water) is stratified by capillarity around the DNA, hydrating it with more nanometric layers, to make it flexible, while at the same time, the capillary action of the EZ water clusters, acts in improving the vital functionality of the cells favoring the exchange by ionic osmosis of the membranes, in particular those related to the oxygenation action exercised by the Mitochondria.(7)

These considerations on the new aspects deriving from the substantial importance of water and the spontaneous emission of bio-quantum light outline a new field of research and development that the research and innovation cluster actively promotes in order to form a better plausible theoretical interpretation in the framework of a substantial paradigm shift to the interaction system of Laser and LED light experimented in the practice of contemporary "Light-Medicine".

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Melanie Ortiz
7 years ago

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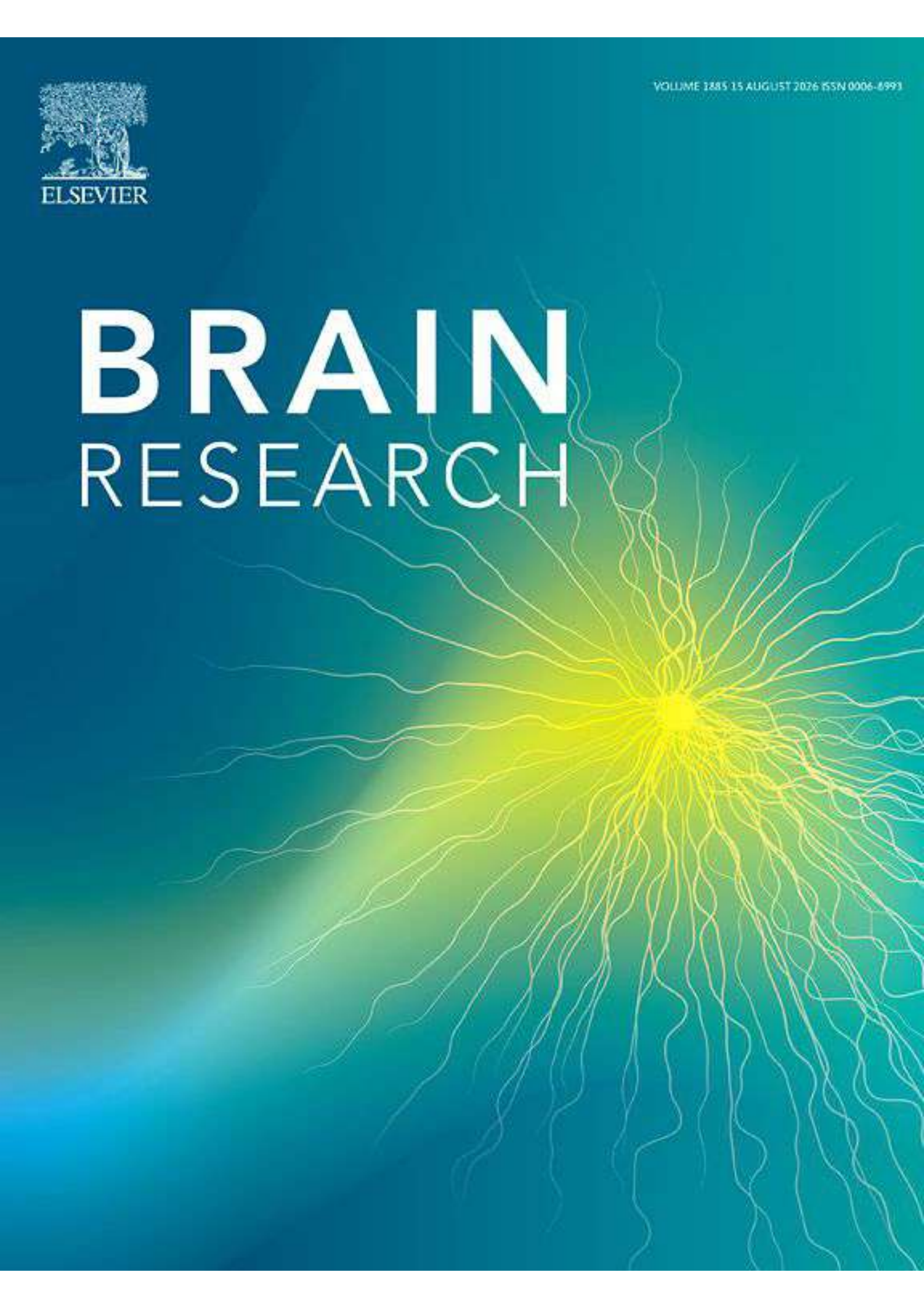

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Window of Opportunity: Menopause, Estrogens and the Brain

Edited by

- Teresa A. Milner
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- Roberta Diaz Brinton

Pages 1-252 (16 March 2011)

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Timing Matters: Menopause and Brain Effects of Ovarian Steroids

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Borobudur revisited: Soy consumption may be associated with better recall in younger, but not in older, rural Indonesian elderly

Eef Hogervorst, Fidiansjah Mursjid, Dewi Priandini, Henry Setyawan, ... Tri Budi Rahardjo

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Research Report

Borobudur revisited: Soy consumption may be associated with better recall in younger, but not in older, rural Indonesian elderly☆

Eef Hogervorst[a,b,*], *Fidiansjah Mursjid*[b], *Dewi Priandini*[b], *Henry Setyawan*[c], *Raden Irawati Ismael*[b], *Stephan Bandelow*[a], *Tri Budi Rahardjo*[b]

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ABSTRACT

Previous reports have suggested that high frequent tofu consumption is associated with worse cognitive function in East Asian elderly. Some studies also found an increased risk of dementia with high tofu consumption in those older than 65 years of age. Tofu and other soy products, such as tempeh, contain high levels of plant estrogens or isoflavones. This study revisited a rural Central Javanese population (56–97 years of age) who were covered by the Borobudur District Health Centers. Data on cognitive performance were available for $n=142$ participants. Results showed positive linear associations of weekly tofu (beta=.22, $p<0.05$) and tempeh (beta=.23, $p<0.01$) consumption with immediate recall, which were significant in those with an average age of 67 years. In those with an average age of 80 years, the earlier reported negative association of tofu with immediate recall was no longer significant. Lifestyle changes (reduction of tofu consumption after dissemination of results) or "healthy survivor effects" may have been responsible for this finding. These findings may be reminiscent of the "Window of Opportunity" theory, which suggests that estrogenic compounds can exert positive effects on verbal memory, but not in older men and women, when no or negative effects of these compounds on brain cells and cognition have been found. Long-term, placebo-controlled treatment studies should investigate whether tempeh, a fermented soybean product that also contains folate, can maintain cognitive function in middle-aged and elderly participants.

☆ Disclosure: We have nothing to disclose and none of the authors has any vested interest in any of the products mentioned in this paper. All authors have contributed to the paper.

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Abbreviations: AD, Alzheimer's disease; E2, estradiol; DR, delayed recall; IR, immediate recall; HT, hormone therapy; HVLT, Hopkins Verbal Learning Test; MMSE, Mini-Mental Status Examination; SD, standard deviation; SES, socioeconomic status; WHIMS, Women's Health Initiative Memory Study

0006-8993/$ – see front matter.
doi:10.1016/j.brainres.2010.10.083

1. Introduction

Previous studies investigating the potential of plant estrogens, such as isoflavones, to protect the aging brain and cognitive function have found contradicting results (see Zhao and Brinton, 2007; Clifford et al., 2009; Hogervorst et al., 2009c; Yesufu et al., in press, for reviews). Because of their chemical similarity to estrogens, isoflavones can exert estrogenic effects on the brain, particularly when endogenous estrogen levels are low (Zhao and Brinton, 2007; Yesufu et al., in press). Soy based products, such as tofu and tempeh, contain high levels of isoflavones (Wang and Murphy, 1994). Although some observational studies have not found an association between cognition and isoflavone intake in persons with low soy consumption (Yesufu et al., in press), high regular consumption of the isoflavone rich tofu paradoxically increased dementia risk in older (>65 years of age) East Asian populations (Rice et al., 2000; White et al., 2000; Hogervorst et al., 2008). In those older than 68 years of age, we found negative linear associations of the potent isoflavone genistein with cognitive function (Hogervorst et al., 2009c).

However, we also found curvilinear associations of genistein with better immediate recall performance in middle-aged (<68 years of age) men and women who were closer to the age of menopause. This suggests that optimal levels of genistein were associated with better memory function in the middle-aged (Hogervorst et al., 2009c). Similarly, randomized controlled treatment studies mainly found positive effects of soy based isoflavones on cognition in women close to the age of menopause, with no or mixed effects in the older population (Yesufu et al., in press). These findings may be reminiscent of the "Window of Opportunity" theory, which is supported by animal and observational data and suggests that effects of estrogens are most prominent when treatment is given close to the age of menopause, when organ systems are still responsive to estrogens' effect (Henderson et al., 2005).

However, this theory does not explain negative effects of estrogens and estrogenic compounds in older participants. Using assays of sufficient sensitivity and controlling for morbidity and medication use affecting sex steroid levels, several investigators reported that women with Alzheimer's disease (AD), the most common type of dementia in the West (Zhao and Brinton, 2007), actually had slightly higher levels of estrogens than controls (Cunningham et al., 2001; Hogervorst et al., 2003; Paoletti et al., 2004). Longitudinal studies also confirmed increased risk for dementia with higher levels of estrogen in elderly women (Geerlings et al., 2006; Ravaglia et al., 2007). In addition, high levels of estrogens in elderly women and men (>65 years of age) are not necessarily associated with better brain function (Yaffe et al., 2002; Den Heijer et al., 2003; Muller et al., 2009; Hogervorst et al., 2010). These data may also be in accordance with the data of the Women's Health Initiative Memory Study (WHIMS) which reported negative effects of estrogen treatment on cognitive function (Espeland et al., 2004) and an increased risk of dementia in women over 65 years of age (Shumaker et al., 2003, 2004).

These negative associations of estrogens and estrogenic compounds (such as genistein, which is abundant in tofu) with brain function in older (>65 years of age) participants may be explained by the "healthy cell bias" hypothesis (Brinton, 2008a,b). This hypothesis is based on cell culture studies which show that brain cells, which are undergoing pathological change (which is more likely to be the case in older neurons), do not actually benefit from an estrogenic environment and are more likely to show accelerated demise in this environment. This is different for healthy neurons, which show benefit from an estrogenic environment. This hypothesis both explains positive effects of estrogens on the brains of younger, middle-aged women and younger (but not older) ovariectomized animals (Gibbs, 2009) and also the negative effects of estrogens and estrogenic compounds on cognition in older women and men.

A caveat is that we also found positive effects of tempeh in the Indonesian study when it was entered in the same statistical model and where it seemed to offset the negative association of high tofu intake with memory (Hogervorst et al., 2008). Tempeh is a fermented whole soybean product which is usually found to contain higher levels of genistein and other isoflavones than tofu (Wang and Murphy, 1994). In addition, due to fermentation with molds, levels of folate are also increased in this food product (Ginting and Arcot, 2004). In our earlier analyses of the Oxford Project to Investigate Memory and Aging data, we found that older women with higher levels of estrogens had a higher risk of dementia (Hogervorst et al., 2003). However, when women also had high levels of serum folate, they did not perform under the cutoff scores for dementia on the Mini-Mental Status Examination (MMSE) (Hogervorst and Smith, 2002). It may thus be the case that folate or other compounds in tempeh protect against potentially negative effects of estrogenic compounds in the elderly.

We revisited rural Central Java where we previously found the strongest negative associations of tofu consumption with immediate recall in those over 68 years of age (Hogervorst et al., 2008) to further investigate the association of different soy products with memory function. On the basis of the previous studies done, we hypothesized that tempeh (because it contains folate) would show protective associations with immediate recall in both the middle-aged (65 years of age and younger) and those who were 66 years of age and older. In separate analyses, tofu was expected to only show this positive association in the middle-aged but to have a negative association with immediate recall in those older than 65 years. Immediate recall was previously shown sensitive to dementia (Hogervorst et al., 2002a), estrogens (Hogervorst et al., 2009b), and soy product consumption (Hogervorst et al., 2008). We also performed a more in depth socioeconomic assessment to investigate whether this mediated the association of soy products with cognition. People of low socioeconomic status were hypothesized to eat more soy (as an animal protein substitute because it is cheaper). However, low socioeconomic status in itself is associated with a faster cognitive decline and an earlier onset of dementia (Karp et al., 2004) and could thus have mediated the association between high soy intake and low memory function.

2. Results

Cognitive data were available of $n=142$ rural participants. Almost half had not attended school, and most others had only obtained primary schooling. Many (45%) still worked as

farmers or laborers and people on average only had 5 household goods or luxuries indicating socioeconomic status, of which the most frequent were electricity (98%), a TV (71%), a radio (57%), livestock (56%), and a bicycle (49%). Socioeconomic status (SES) as assessed by these parameters would, on average, thus be low for the villagers, but data distribution was not skewed, showing a good spread in goods available to villagers (Table 1).

Median and mean tempeh and tofu consumption was daily (i.e. 7 times a week, SD=5), ranging from never to 3 times a day. In comparison, only 18% of this sample ate fruit "regularly" and, of these participants, only half did so on a daily basis ($n=15$). The average weekly consumption of fruit was only 2 pieces of fruit (SD=2.5), despite availability in the area.

Spearman's rank correlation suggested that higher weekly tempeh consumption was associated with better immediate recall, as measured using the 3 trials of the Hopkins Verbal Learning Test (HVLT IR, rho=.19; $p<0.05$), with a similar trend for tofu (rho=.16, $p=0.06$; see Fig. 1). Regular (but not weekly) fruit consumption was also associated with better immediate recall (HVLT IR, rho=.18; $p<0.05$). Delayed recall as measured by using the HVLT was not associated with intake of any of the foods ($p>0.28$).

2.1. Other factors associated with food intake

Fruit intake, when calculated in consumption per week, was only positively associated with total tempeh and tofu intake per week (rho=.30 and =.41, respectively; $p<0.001$). Those who ate more tempeh also ate more tofu (rho=.82, $p<0.001$). This suggests dietary patterns (healthy patterns or perhaps patterns reflecting abundance). Unfortunately, we did not have data on overall caloric intake to calculate the proportionate intake of these healthy foods.

Tempeh and tofu consumption was higher in more educated people (rho=.24 and .19, respectively; $p<0.05$) and,

Table 1 – Demographic characteristics of rural Central Javanese participants.

Variable	Percentage or mean (SD)	Range
Sex (women)	61%	
Ethnicity (Javanese)	100%	
Religion (Muslim)	100%	
Age (mean and median)	73 (SD=8)	56–97
Household goods (SES)	4.6 (SD=2.7)	0–15 of 18
Occupation		
Not working/retired	38%	
Farmers	30.3%	
Laborers	14.8%	
Business/entrepreneur	12%	
Other	5%	
Education		
No education	48%	
Part of primary school	25%	
Primary school finished	14%	
Secondary school	8.5%	
High school	3.5%	
HVLT total immediate recall	14 words (SD=7.12)	0–36
HVLT delayed recall (max 12)	5 words (SD=2.92)	0–12

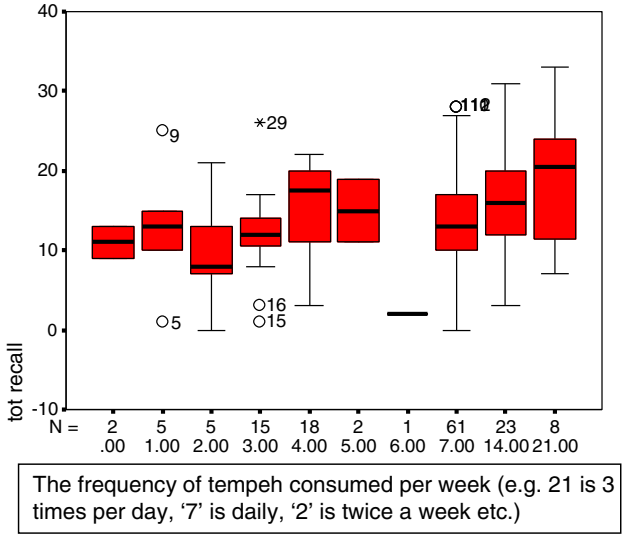

Fig. 1 – Box plots (with the bottom and top of the box being the 25th and 75th percentile (the lower and upper quartiles, respectively), and the band near the middle being the median, with outliers) of the total immediate recall performance on the HVLT ("tot recall," in number of words recalled over 3 trials, with a maximum of 36 words recalled in total) and the frequency of tempeh consumed per week on the y-axis, with n referring to numbers of participants per box plot.

(in contrast to our expectations), tofu was also more likely to be consumed by those of higher socioeconomic status (SES), as assessed by the number of household goods or luxuries (rho=.18, $p<0.05$). Box plots showed not only that those who were farmers or laborers had a maximum daily median intake of tofu but also that more than half of the entrepreneurs were able to consume more than that and that these ate tofu 2 or 3 times a day. A similar trend ($p=0.07$) existed for tempeh with SES, but this did not exist for fruit consumption. Older people were in general less well-educated (rho=–.28, $p<0.001$) and had fewer household goods or luxuries (rho=–.29, $p<0.001$). Better-educated people also had more household goods (rho=.36, $p<0.0001$).

2.2. Other factors associated with immediate recall

Immediate recall was associated with having had more education (rho=.41, $p<0.001$) and having more household goods (rho=.18, $p<0.05$). Those who were older had worse immediate recall (HVLT IR rho=–.39; $p<0.001$). There was no gender difference on immediate recall performance, perhaps because there was no age difference between men and women of this cohort or because women had received, on average, less education ($p<0.001$) than men.

Using linear regression indicated that there were significant associations of tofu (beta=.22, $p<0.05$) and tempeh[1] (beta=.23, $p<0.01$; see Fig. 1) with immediate recall performance (HVLT IR total recall but not DR), which was independent of SES and weekly fruit intake, which were both non-significant. However,

[1] Tempeh and tofu were entered separately, as there was significant co-linearity.

associations between soy consumption and immediate recall were no longer significant when age, sex, and education were also entered in the model. A trend remained for weekly tofu consumption (beta=.14, $p=.10$), to be associated with better immediate recall, when the other covariates were entered (age: beta=–.29, $p<0.001$; education: beta=.33, $p<0.0001$; gender: $p=.78$; SES, $p=.97$). In this model, regular fruit consumption (beta=.15, $p<0.05$) was also independently associated with better immediate recall.

Delayed recall showed no associations with soy product consumption or fruit intake using similar analyses.

In analyses stratified for median age, in those who were younger than 73 years of age ($n=74$; mean age, 67 years), weekly tofu consumption was a significant predictor of immediate recall (beta=.21, $p=.05$; and a similar trend existed for tempeh: beta=.19, $p=0.09$). This association remained significant after addition of the covariates. Of these covariates, only education (beta=.47, $p<0.001$) was an independent predictor of better performance. Age, SES, and gender were non-significant contributors to this model. There was also a trend for regular (but not weekly) fruit consumption (beta=.18, $p=.09$) to be associated with better performance in those younger than 73 years.

However, these associations were not replicated in those who were older than 73 years of age ($n=68$; mean age, 80 years), where age was the only significant predictor of low immediate recall (beta=–.40, $p=0.006$). However, in this group, the direction of the association between tofu and immediate recall was negative (but this did not reach significance).

Using a different stratification by investigating those participants who were younger than 66 years of age (which included 20% of the sample and who were considered to be "middle-aged" to investigate the "Window of Opportunity" hypothesis) versus those who were older, tempeh (beta=.47, $p<0.05$) and tofu (beta=.49, $p<0.05$) both had a significant association with immediate recall in unadjusted models but again only in those younger than 66 years of age and not in the older participants or when analyses were adjusted for covariates. Entering curvilinear terms did not result in better fits within the models, indicating no optimal levels of soy consumption for optimal immediate recall. Delayed recall again showed no associations with soy product consumption using similar analyses.

3. Discussion

Results reflect earlier findings of regular consumption of soy products and fruit having positive associations with immediate recall in those with an average age of 67 years of age (<73 years of age) but not in those with an average age of 80 years of age. In this study, we further investigated whether low socioeconomic status mediated the negative associations found in earlier studies between frequent tofu consumption and cognition. However, the opposite was found with rural elderly who had more household goods and luxuries as indicators of socioeconomic status eating more soy products than those who had less. Socioeconomic status also did not mediate the associations found.

Delayed recall, which is considered by some to be a more sensitive measure of impeding dementia, was in the present and earlier studies not associated with soy consumption. With an average of 5 words recalled, resolution was low for these analyses. In our earlier work, the total immediate recall showed similar sensitivity and specificity for dementia as the delayed recall (Hogervorst et al., 2002a) but immediate recall has a wider spread of data, creating better resolution for data analyses. Similar test sensitivity issues were also seen for estrogenic compounds where not all verbal memory tests are equally affected (Sherwin, 1994), and meta-analyses indicated that immediate recall of word lists seemed most sensitive to effects of estrogen treatment than delayed recall (Hogervorst et al., 2002b).

This study thus extends and confirms our earlier work suggesting that tempeh had protective associations with immediate recall, although the earlier reported negative associations of high tofu consumption with immediate recall were not significant in the present study. This was possibly because of a smaller sample size in this cohort as compared to that of the previous study ($n=214$) (Hogervorst et al., 2008). Alternatively, lifestyle changes may be responsible for these findings as all participants had been briefed about the negative associations of tofu and memory found after data analyses. Post hoc analyses showed that, of those investigated and followed up in this paper, only 21% ate tofu more than once a day, whereas at baseline testing, this was almost double (38%), suggesting that more people had cut down on tofu consumption (or alternatively, that more of those with high consumption had died earlier). In 2009, more people ate tofu just once a day (48%) compared to 30% at baseline in 2007.

Another hypothesis from our earlier work (Hogervorst et al., 2008) was that negative associations of tofu with immediate recall and dementia risk in those over 68 years (in that paper) were found because of addition of formaldehyde to tofu to preserve its freshness. Several factories in Jakarta had apparently sold tofu laced with this highly neurotoxic compound to traditional markets (see The Jakarta Post 07/31/2009 and 09/11/2008), and according to the Department of Public Health, this practice was widespread. However, when we tested for this in 2009 in various tofu samples of shops and vendors around Jakarta, we could not find evidence of addition of formaldehyde to tofu (Aryani, 2009; PhD thesis, Universitas Indonesia). It is also unclear whether laced tofu is still sold in rural Central Java and more research needs to be done to investigate this association. This practice does not occur in tempeh, which can be kept fresh for longer periods due to fermentation of this product.

Using curvilinear terms, we did not find optimal levels of soy consumption in those with an average age of 67 years, although in this age group, we earlier had reported optimal levels of salivary genistein, the most active estrogenic compound (Hogervorst et al., 2009a). The Study of Women's Health Across the Nation (SWAN) did not find optimal calculated levels of genistein and daidzein in their East Asian middle-aged women (Huang et al., 2006). However, in this study, isoflavone levels were inferred from food frequency questionnaires, and so, these were not measured directly, similar to the present study where we only included food frequency data. The authors also used tertiles to investigate optimal genistein and daidzein levels, rather than using continuous variables as we did earlier. The SWAN also did not report *weekly* soy intake as was done in the other and present studies reporting significant associations with

cognitive function. For instance, in the Honolulu-Asia Aging Study, Japanese-American men and women who consumed tofu more than twice a week in midlife and in old age had a higher risk for dementia, lower brain weight, and more ventricular enlargement (White et al., 2000). In the KAME project (Rice et al., 2000), Japanese-American women over 65 years of age who consumed tofu more than 3 days a week had lower cognitive performance. In stratified analyses, this remained significant only for those women taking hormone therapy, suggesting a cumulative negative effect of estrogenic compounds on cognition. In the present Javanese cohort studied, none of the women took hormone therapy, but 5 reported to have not undergone menopause yet. However, post hoc analyses of soy product consumption and its association with immediate recall performance excluding these participants were not substantially different from those currently presented in the Results section. Lastly, in the KAME project (Rice et al., 2000), at 2 year follow-up, all those who had moderate tofu consumption had most cognitive improvement over time. Where optimal soy intake lies at what age for maintenance of cognitive performance over time and how this is associated with other dietary intakes and possibly genetic profiles relating to sex steroid metabolism and synthesis (Hogervorst and Bandelow, 2007) remains to be further investigated. Other studies including elderly women with Western diets with low average soy intake have not found any associations with cognition (Yesufu et al., in press), which may further suggest that dosage and age play an important role rather than consumption per se.

As there were also positive associations of both soy products with higher fruit consumption, healthy dietary patterns may have confounded the positive associations reported in the present study. However, positive associations of regular fruit consumption with immediate recall were independent of those of tofu and, in separate analyses, also of tempeh consumption. On the other hand, weekly consumption of fruit had no significant association. This may be because of low resolution in these data as, of the total cohort, only 15 elderly ate fruit daily, which had previously been found to be a protective factor against dementia (Hogervorst et al., 2008). There may be a further role here for public health messages promoting higher fruit intake.

Limitations of the present study are that we did not include medical examination data in this study and could therefore not control for medication use and morbidity (including dementia), which could have affected results. This could be a reason why no associations were found of food intake in those with an average age of 80 years in this study who may have been more at risk for morbidity (e.g. chronic infectious disease), which could have potentially overruled smaller protective effects of nutrition on cognitive function. However, all participants were community-dwelling and fit enough to travel by car without apparent acute systemic morbidity. Survivor effects, with only the very healthy surviving to an old age, limit generalization of results, and this will be investigated in detail using the follow-up data. Soy products are thought to protect against many age related morbidities, such as cancer and heart disease, which may have also explained positive associations with immediate recall function, rather than through the reported positive effects of isoflavones on brain cells directly (Zhao et al., 2002; Zhao and Brinton, 2007). Other limitations in the present study are the reliance on self report which could confound associations. Those with memory issues would be more likely to underreport soy consumption, similar to data found for hormone treatment (Petitti et al., 2002). However, all carers were present and confirmed answers were given by participants. Where there was discrepancy, the answer of the carer was used. In addition, positive associations were mainly found in those with an average age of 67 years, where recall issues would not be considered to be a major confounding issue, as dementia prevalence starts to increase mainly after 65 years of age.

Data may reflect findings from estrogen treatment where positive effects were most apparent in those who are middle-aged but where no or negative effects were seen in those over 65 years of age (Hogervorst et al., 2009b). Using isoflavone supplements after the menopause has become more popular as an alternative to combat menopausal symptoms (such as hot flashes, night sweats, and cognitive complaints). Short-, but not long-term, treatment studies have shown positive effects of isoflavones on cognitive function (File et al., 2005; Gleason et al., 2009; but see Maki et al., 2009). These data may have a similar pattern to that of estrogen treatment, which probably only has a limited duration positive effect on cognition. Meta-analyses showed positive effects of estrogen treatment on some cognitive tests but only up to 2–3 months (Hogervorst et al., 2002b; Hogervorst et al., 2009b). It may be that different estrogen or soy treatment regimes with intermittent exposure to limit desensitization to continuous treatment could prolong positive effects (Hogervorst, 2009c). Alternatively, genetic subgroups could exist for whom estrogen treatment is not indicated as it increases dementia risk due to formation and accumulation of toxic estrogenic metabolites (Hogervorst et al., 2009a). Long-term, well-controlled, large treatment studies into the effects of soy products on the brain are thus necessary. One relatively recent treatment study showed not only some positive effects of a high-dose 6 month treatment with 100 mg soy isoflavones but also some of placebo in older men and women over 62 years of age (Gleason et al., 2009). In sum, despite not finding significant negative associations in this study, it should be noted that not all that is “natural” is a priori more healthy especially for those over 65 years of age, for whom long-term, high-dose, continuous exposure to estrogenic compounds may not be indicated to preserve brain integrity.

4. Experimental procedures

Rural, community-dwelling elderly of Central Java were included in this survey. Participants had to be over 56 years of age to be included and the villages they resided in had to be covered by the local community health districts of rural Borobudur and Salaam (a 2 h drive from Yogyakarta). Of those included, 54% ($n=115$) could be cognitively assessed for follow-up assessment in April 2009 (of the $n=214$ assessed at baseline in 2006; see Hogervorst et al., 2008). This paper concerns the cross sectional data of the total rolling cohort collected in April 2009, which also included novel participants ($n=33$) who had reached the age of 56 years in 2009. Data on tofu consumption were available for $n=151$. Ethical approval from Loughborough University and the University of Indonesia, governmental permits, and informed

consent were obtained before data collection. Participants had been informed of the research during their visits to the health clinics weeks before testing. They had been made aware that non-participation would in no way affect their treatment at the health center and that this was not obligatory. However, because of the excellent relationship between health center staff and the elderly, the willingness to participate in research was high. Participants were collected by car to travel to the research setting at an agreed date with their carer (only 2 came alone) who also signed the informed consent. This approach had a 96% response rate of those contacted, as only 7 participants of those contacted refused to be tested because of fatigue or frailty on the test day. All others consented to participate. The research was carried out in 1 day in the 5 rooms of the home of one of the administrators of the integrated health unit (Pos Pelayanan Terpadu/Posyandu), which was quiet and which had no distractions. Assessments included cognitive testing and a survey consisting of standardized interview questions, which were carried out by trained researchers.

4.1. *Demographics and lifestyle variables*

The survey consisted of questions about demographics (e.g. age, education, occupation), including an 18-item household goods and luxury questionnaire (electronics, transportation, luxury items) as an indicator of socioeconomic status, and lifestyle variables (e.g. activity, diet, and health). Diet was assessed using a food frequency questionnaire (see Hogervorst et al., 2008; Frankenfeld et al., 2004), which included questions about "regular" consumption of products (yes or no), which were followed by questions on the frequency of daily, weekly, or monthly consumption of these products, such as tempeh and tofu. These questions were all transformed into weekly consumption (e.g. eating tofu once daily was calculated as 7 times a week, eating tofu 3 times a day for 7 days a week was calculated as tofu consumption 21 times a week, whereas once a month was calculated as 0.25 times a week, etc.).

4.2. *Medical examination and cognitive assessments*

A medical examination and a health resume including a carer's report were based on the Cambridge Mental Disorder of the Elderly Examination (CAMDEX; Roth et al., 1988). This also included a psychiatric assessment and a depression scale. Depression was not considered to be severe enough to interfere with cognitive function in any of the participants. None of the participants displayed symptoms of anxiety or other psychiatric morbidity, but one participant with psychosis was excluded. Alcohol was not consumed by any of the participants, all of whom were Muslim.

The modified Hopkins Verbal Learning Test (HVLT; Brandt, 1991; Hogervorst et al., 2008) was administered by trained research assistants. The HVLT is a short (5–10 min) test that is well tolerated. It is a memory test consisting of 12 words which belong to 3 categories (human shelter, precious stones, and animals for list A). The modified test was adapted to local knowledge (e.g. some of the semi precious stones were not known by the local population in a pilot study). It had been translated into Bahasa Indonesia and Javanese and was then back-translated to English by an independent party successfully. Outcomes include the total immediate recall (IR, which assesses learning ability over 3 trials) and the delayed recall (DR), which is done after a 20–30 min interval during which other tests are done or questionnaires are filled in. Memory is one of the earliest functions to decline in dementia (McKhann et al., 1984; American Psychiatric Association, 1994). The total immediate recall of the HVLT had high sensitivity and specificity for dementia when assessed in different cohorts in the US and UK (Brandt, 1991; Hogervorst et al., 2002a; De Jager et al., 2003). Immediate recall of word lists is also sensitive to effects of estrogenic compounds (Hogervorst et al., 2002b) and therefore was the main outcome parameter we reported on in this paper. Test performance distribution in rural Java was normal and was similar to that of a similar aged population in Oxfordshire (Hogervorst et al., 2002a).

4.3. *Statistical analyses*

All data were checked for missing values and potential outliers. Non-parametric Spearman's correlation analyses were performed to assess associations between variables. Linear regression analyses were carried out to investigate whether tofu and tempeh intake was associated with immediate recall function, independent of socioeconomic status (as assessed by household goods) and regular intake of fruits (to control for having an overall healthier dietary pattern) in a first step, and age, education, and gender in a second step. We stratified analyses for age using two different methods. One used a median split, the other investigated the subgroup of middle-aged participants who were younger than 66 years of age (who were considered to be "middle-aged") versus the other group who were older to test the "Window of Opportunity" hypothesis. All analyses were performed in SPSS 17.0 using a p-value of 0.05.

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Research Report

Borobudur revisited: Soy consumption may be associated with better recall in younger, but not in older, rural Indonesian elderly☆

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ABSTRACT

Previous reports have suggested that high frequent tofu consumption is associated with worse cognitive function in East Asian elderly. Some studies also found an increased risk of dementia with high tofu consumption in those older than 65 years of age. Tofu and other soy products, such as tempeh, contain high levels of plant estrogens or isoflavones. This study revisited a rural Central Javanese population (56–97 years of age) who were covered by the Borobudur District Health Centers. Data on cognitive performance were available for $n=142$ participants. Results showed positive linear associations of weekly tofu (beta=.22, $p<0.05$) and tempeh (beta=.23, $p<0.01$) consumption with immediate recall, which were significant in those with an average age of 67 years. In those with an average age of 80 years, the earlier reported negative association of tofu with immediate recall was no longer significant. Lifestyle changes (reduction of tofu consumption after dissemination of results) or "healthy survivor effects" may have been responsible for this finding. These findings may be reminiscent of the "Window of Opportunity" theory, which suggests that estrogenic compounds can exert positive effects on verbal memory, but not in older men and women, when no or negative effects of these compounds on brain cells and cognition have been found. Long-term, placebo-controlled treatment studies should investigate whether tempeh, a fermented soybean product that also contains folate, can maintain cognitive function in middle-aged and elderly participants.

☆ Disclosure: We have nothing to disclose and none of the authors has any vested interest in any of the products mentioned in this paper. All authors have contributed to the paper.

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Abbreviations: AD, Alzheimer's disease; E2, estradiol; DR, delayed recall; IR, immediate recall; HT, hormone therapy; HVLT, Hopkins Verbal Learning Test; MMSE, Mini-Mental Status Examination; SD, standard deviation; SES, socioeconomic status; WHIMS, Women's Health Initiative Memory Study

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1. Introduction

Previous studies investigating the potential of plant estrogens, such as isoflavones, to protect the aging brain and cognitive function have found contradicting results (see Zhao and Brinton, 2007; Clifford et al., 2009; Hogervorst et al., 2009c; Yesufu et al., in press, for reviews). Because of their chemical similarity to estrogens, isoflavones can exert estrogenic effects on the brain, particularly when endogenous estrogen levels are low (Zhao and Brinton, 2007; Yesufu et al., in press). Soy based products, such as tofu and tempeh, contain high levels of isoflavones (Wang and Murphy, 1994). Although some observational studies have not found an association between cognition and isoflavone intake in persons with low soy consumption (Yesufu et al., in press), high regular consumption of the isoflavone rich tofu paradoxically increased dementia risk in older (>65 years of age) East Asian populations (Rice et al., 2000; White et al., 2000; Hogervorst et al., 2008). In those older than 68 years of age, we found negative linear associations of the potent isoflavone genistein with cognitive function (Hogervorst et al., 2009c).

However, we also found curvilinear associations of genistein with better immediate recall performance in middle-aged (<68 years of age) men and women who were closer to the age of menopause. This suggests that optimal levels of genistein were associated with better memory function in the middle-aged (Hogervorst et al., 2009c). Similarly, randomized controlled treatment studies mainly found positive effects of soy based isoflavones on cognition in women close to the age of menopause, with no or mixed effects in the older population (Yesufu et al., in press). These findings may be reminiscent of the "Window of Opportunity" theory, which is supported by animal and observational data and suggests that effects of estrogens are most prominent when treatment is given close to the age of menopause, when organ systems are still responsive to estrogens' effect (Henderson et al., 2005).

However, this theory does not explain negative effects of estrogens and estrogenic compounds in older participants. Using assays of sufficient sensitivity and controlling for morbidity and medication use affecting sex steroid levels, several investigators reported that women with Alzheimer's disease (AD), the most common type of dementia in the West (Zhao and Brinton, 2007), actually had slightly higher levels of estrogens than controls (Cunningham et al., 2001; Hogervorst et al., 2003; Paoletti et al., 2004). Longitudinal studies also confirmed increased risk for dementia with higher levels of estrogen in elderly women (Geerlings et al., 2006; Ravaglia et al., 2007). In addition, high levels of estrogens in elderly women and men (>65 years of age) are not necessarily associated with better brain function (Yaffe et al., 2002; Den Heijer et al., 2003; Muller et al., 2009; Hogervorst et al., 2010). These data may also be in accordance with the data of the Women's Health Initiative Memory Study (WHIMS) which reported negative effects of estrogen treatment on cognitive function (Espeland et al., 2004) and an increased risk of dementia in women over 65 years of age (Shumaker et al., 2003, 2004).

These negative associations of estrogens and estrogenic compounds (such as genistein, which is abundant in tofu) with brain function in older (>65 years of age) participants may be explained by the "healthy cell bias" hypothesis (Brinton, 2008a,b). This hypothesis is based on cell culture studies which show that brain cells, which are undergoing pathological change (which is more likely to be the case in older neurons), do not actually benefit from an estrogenic environment and are more likely to show accelerated demise in this environment. This is different for healthy neurons, which show benefit from an estrogenic environment. This hypothesis both explains positive effects of estrogens on the brains of younger, middle-aged women and younger (but not older) ovariectomized animals (Gibbs, 2009) and also the negative effects of estrogens and estrogenic compounds on cognition in older women and men.

A caveat is that we also found positive effects of tempeh in the Indonesian study when it was entered in the same statistical model and where it seemed to offset the negative association of high tofu intake with memory (Hogervorst et al., 2008). Tempeh is a fermented whole soybean product which is usually found to contain higher levels of genistein and other isoflavones than tofu (Wang and Murphy, 1994). In addition, due to fermentation with molds, levels of folate are also increased in this food product (Ginting and Arcot, 2004). In our earlier analyses of the Oxford Project to Investigate Memory and Aging data, we found that older women with higher levels of estrogens had a higher risk of dementia (Hogervorst et al., 2003). However, when women also had high levels of serum folate, they did not perform under the cutoff scores for dementia on the Mini-Mental Status Examination (MMSE) (Hogervorst and Smith, 2002). It may thus be the case that folate or other compounds in tempeh protect against potentially negative effects of estrogenic compounds in the elderly.

We revisited rural Central Java where we previously found the strongest negative associations of tofu consumption with immediate recall in those over 68 years of age (Hogervorst et al., 2008) to further investigate the association of different soy products with memory function. On the basis of the previous studies done, we hypothesized that tempeh (because it contains folate) would show protective associations with immediate recall in both the middle-aged (65 years of age and younger) and those who were 66 years of age and older. In separate analyses, tofu was expected to only show this positive association in the middle-aged but to have a negative association with immediate recall in those older than 65 years. Immediate recall was previously shown sensitive to dementia (Hogervorst et al., 2002a), estrogens (Hogervorst et al., 2009b), and soy product consumption (Hogervorst et al., 2008). We also performed a more in depth socioeconomic assessment to investigate whether this mediated the association of soy products with cognition. People of low socioeconomic status were hypothesized to eat more soy (as an animal protein substitute because it is cheaper). However, low socioeconomic status in itself is associated with a faster cognitive decline and an earlier onset of dementia (Karp et al., 2004) and could thus have mediated the association between high soy intake and low memory function.

2. Results

Cognitive data were available of $n=142$ rural participants. Almost half had not attended school, and most others had only obtained primary schooling. Many (45%) still worked as

farmers or laborers and people on average only had 5 household goods or luxuries indicating socioeconomic status, of which the most frequent were electricity (98%), a TV (71%), a radio (57%), livestock (56%), and a bicycle (49%). Socioeconomic status (SES) as assessed by these parameters would, on average, thus be low for the villagers, but data distribution was not skewed, showing a good spread in goods available to villagers (Table 1).

Median and mean tempeh and tofu consumption was daily (i.e. 7 times a week, SD=5), ranging from never to 3 times a day. In comparison, only 18% of this sample ate fruit "regularly" and, of these participants, only half did so on a daily basis ($n=15$). The average weekly consumption of fruit was only 2 pieces of fruit (SD=2.5), despite availability in the area.

Spearman's rank correlation suggested that higher weekly tempeh consumption was associated with better immediate recall, as measured using the 3 trials of the Hopkins Verbal Learning Test (HVLT IR, rho=.19; $p<0.05$), with a similar trend for tofu (rho=.16, $p=0.06$; see Fig. 1). Regular (but not weekly) fruit consumption was also associated with better immediate recall (HVLT IR, rho=.18; $p<0.05$). Delayed recall as measured by using the HVLT was not associated with intake of any of the foods ($p>0.28$).

2.1. Other factors associated with food intake

Fruit intake, when calculated in consumption per week, was only positively associated with total tempeh and tofu intake per week (rho=.30 and =.41, respectively; $p<0.001$). Those who ate more tempeh also ate more tofu (rho=.82, $p<0.001$). This suggests dietary patterns (healthy patterns or perhaps patterns reflecting abundance). Unfortunately, we did not have data on overall caloric intake to calculate the proportionate intake of these healthy foods.

Tempeh and tofu consumption was higher in more educated people (rho=.24 and .19, respectively; $p<0.05$) and,

Table 1 – Demographic characteristics of rural Central Javanese participants.

Variable	Percentage or mean (SD)	Range
Sex (women)	61%	
Ethnicity (Javanese)	100%	
Religion (Muslim)	100%	
Age (mean and median)	73 (SD=8)	56–97
Household goods (SES)	4.6 (SD=2.7)	0–15 of 18
Occupation		
Not working/retired	38%	
Farmers	30.3%	
Laborers	14.8%	
Business/entrepreneur	12%	
Other	5%	
Education		
No education	48%	
Part of primary school	25%	
Primary school finished	14%	
Secondary school	8.5%	
High school	3.5%	
HVLT total immediate recall	14 words (SD=7.12)	0–36
HVLT delayed recall (max 12)	5 words (SD=2.92)	0–12

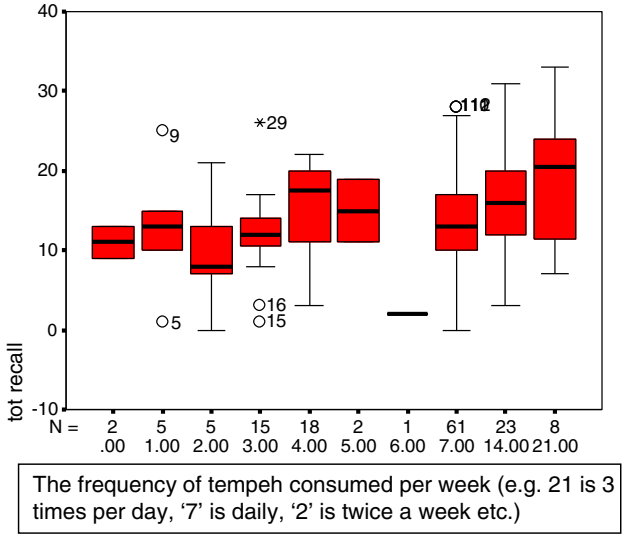

Fig. 1 – Box plots (with the bottom and top of the box being the 25th and 75th percentile (the lower and upper quartiles, respectively), and the band near the middle being the median, with outliers) of the total immediate recall performance on the HVLT ("tot recall," in number of words recalled over 3 trials, with a maximum of 36 words recalled in total) and the frequency of tempeh consumed per week on the y-axis, with n referring to numbers of participants per box plot.

(in contrast to our expectations), tofu was also more likely to be consumed by those of higher socioeconomic status (SES), as assessed by the number of household goods or luxuries (rho=.18, $p<0.05$). Box plots showed not only that those who were farmers or laborers had a maximum daily median intake of tofu but also that more than half of the entrepreneurs were able to consume more than that and that these ate tofu 2 or 3 times a day. A similar trend ($p=0.07$) existed for tempeh with SES, but this did not exist for fruit consumption. Older people were in general less well-educated (rho=–.28, $p<0.001$) and had fewer household goods or luxuries (rho=–.29, $p<0.001$). Better-educated people also had more household goods (rho=.36, $p<0.0001$).

2.2. Other factors associated with immediate recall

Immediate recall was associated with having had more education (rho=.41, $p<0.001$) and having more household goods (rho=.18, $p<0.05$). Those who were older had worse immediate recall (HVLT IR rho=–.39; $p<0.001$). There was no gender difference on immediate recall performance, perhaps because there was no age difference between men and women of this cohort or because women had received, on average, less education ($p<0.001$) than men.

Using linear regression indicated that there were significant associations of tofu (beta=.22, $p<0.05$) and tempeh[1] (beta=.23, $p<0.01$; see Fig. 1) with immediate recall performance (HVLT IR total recall but not DR), which was independent of SES and weekly fruit intake, which were both non-significant. However,

[1] Tempeh and tofu were entered separately, as there was significant co-linearity.

associations between soy consumption and immediate recall were no longer significant when age, sex, and education were also entered in the model. A trend remained for weekly tofu consumption (beta=.14, p=.10), to be associated with better immediate recall, when the other covariates were entered (age: beta=–.29, p<0.001; education: beta=.33, p<0.0001; gender: p=.78; SES, p=.97). In this model, regular fruit consumption (beta=.15, p<0.05) was also independently associated with better immediate recall.

Delayed recall showed no associations with soy product consumption or fruit intake using similar analyses.

In analyses stratified for median age, in those who were younger than 73 years of age (n=74; mean age, 67 years), weekly tofu consumption was a significant predictor of immediate recall (beta=.21, p=.05; and a similar trend existed for tempeh: beta=.19, p=0.09). This association remained significant after addition of the covariates. Of these covariates, only education (beta=.47, p<0.001) was an independent predictor of better performance. Age, SES, and gender were non-significant contributors to this model. There was also a trend for regular (but not weekly) fruit consumption (beta=.18, p=.09) to be associated with better performance in those younger than 73 years.

However, these associations were not replicated in those who were older than 73 years of age (n=68; mean age, 80 years), where age was the only significant predictor of low immediate recall (beta=–.40, p=0.006). However, in this group, the direction of the association between tofu and immediate recall was negative (but this did not reach significance).

Using a different stratification by investigating those participants who were younger than 66 years of age (which included 20% of the sample and who were considered to be "middle-aged" to investigate the "Window of Opportunity" hypothesis) versus those who were older, tempeh (beta=.47, p<0.05) and tofu (beta=.49, p<0.05) both had a significant association with immediate recall in unadjusted models but again only in those younger than 66 years of age and not in the older participants or when analyses were adjusted for covariates. Entering curvilinear terms did not result in better fits within the models, indicating no optimal levels of soy consumption for optimal immediate recall. Delayed recall again showed no associations with soy product consumption using similar analyses.

3. Discussion

Results reflect earlier findings of regular consumption of soy products and fruit having positive associations with immediate recall in those with an average age of 67 years of age (<73 years of age) but not in those with an average age of 80 years of age. In this study, we further investigated whether low socioeconomic status mediated the negative associations found in earlier studies between frequent tofu consumption and cognition. However, the opposite was found with rural elderly who had more household goods and luxuries as indicators of socioeconomic status eating more soy products than those who had less. Socioeconomic status also did not mediate the associations found.

Delayed recall, which is considered by some to be a more sensitive measure of impeding dementia, was in the present and earlier studies not associated with soy consumption. With an average of 5 words recalled, resolution was low for these analyses. In our earlier work, the total immediate recall showed similar sensitivity and specificity for dementia as the delayed recall (Hogervorst et al., 2002a) but immediate recall has a wider spread of data, creating better resolution for data analyses. Similar test sensitivity issues were also seen for estrogenic compounds where not all verbal memory tests are equally affected (Sherwin, 1994), and meta-analyses indicated that immediate recall of word lists seemed most sensitive to effects of estrogen treatment than delayed recall (Hogervorst et al., 2002b).

This study thus extends and confirms our earlier work suggesting that tempeh had protective associations with immediate recall, although the earlier reported negative associations of high tofu consumption with immediate recall were not significant in the present study. This was possibly because of a smaller sample size in this cohort as compared to that of the previous study (n=214) (Hogervorst et al., 2008). Alternatively, lifestyle changes may be responsible for these findings as all participants had been briefed about the negative associations of tofu and memory found after data analyses. Post hoc analyses showed that, of those investigated and followed up in this paper, only 21% ate tofu more than once a day, whereas at baseline testing, this was almost double (38%), suggesting that more people had cut down on tofu consumption (or alternatively, that more of those with high consumption had died earlier). In 2009, more people ate tofu just once a day (48%) compared to 30% at baseline in 2007.

Another hypothesis from our earlier work (Hogervorst et al., 2008) was that negative associations of tofu with immediate recall and dementia risk in those over 68 years (in that paper) were found because of addition of formaldehyde to tofu to preserve its freshness. Several factories in Jakarta had apparently sold tofu laced with this highly neurotoxic compound to traditional markets (see The Jakarta Post 07/31/2009 and 09/11/2008), and according to the Department of Public Health, this practice was widespread. However, when we tested for this in 2009 in various tofu samples of shops and vendors around Jakarta, we could not find evidence of addition of formaldehyde to tofu (Aryani, 2009; PhD thesis, Universitas Indonesia). It is also unclear whether laced tofu is still sold in rural Central Java and more research needs to be done to investigate this association. This practice does not occur in tempeh, which can be kept fresh for longer periods due to fermentation of this product.

Using curvilinear terms, we did not find optimal levels of soy consumption in those with an average age of 67 years, although in this age group, we earlier had reported optimal levels of salivary genistein, the most active estrogenic compound (Hogervorst et al., 2009a). The Study of Women's Health Across the Nation (SWAN) did not find optimal calculated levels of genistein and daidzein in their East Asian middle-aged women (Huang et al., 2006). However, in this study, isoflavone levels were inferred from food frequency questionnaires, and so, these were not measured directly, similar to the present study where we only included food frequency data. The authors also used tertiles to investigate optimal genistein and daidzein levels, rather than using continuous variables as we did earlier. The SWAN also did not report *weekly* soy intake as was done in the other and present studies reporting significant associations with

cognitive function. For instance, in the Honolulu-Asia Aging Study, Japanese-American men and women who consumed tofu more than twice a week in midlife and in old age had a higher risk for dementia, lower brain weight, and more ventricular enlargement (White et al., 2000). In the KAME project (Rice et al., 2000), Japanese-American women over 65 years of age who consumed tofu more than 3 days a week had lower cognitive performance. In stratified analyses, this remained significant only for those women taking hormone therapy, suggesting a cumulative negative effect of estrogenic compounds on cognition. In the present Javanese cohort studied, none of the women took hormone therapy, but 5 reported to have not undergone menopause yet. However, post hoc analyses of soy product consumption and its association with immediate recall performance excluding these participants were not substantially different from those currently presented in the Results section. Lastly, in the KAME project (Rice et al., 2000), at 2 year follow-up, all those who had moderate tofu consumption had most cognitive improvement over time. Where optimal soy intake lies at what age for maintenance of cognitive performance over time and how this is associated with other dietary intakes and possibly genetic profiles relating to sex steroid metabolism and synthesis (Hogervorst and Bandelow, 2007) remains to be further investigated. Other studies including elderly women with Western diets with low average soy intake have not found any associations with cognition (Yesufu et al., in press), which may further suggest that dosage and age play an important role rather than consumption per se.

As there were also positive associations of both soy products with higher fruit consumption, healthy dietary patterns may have confounded the positive associations reported in the present study. However, positive associations of regular fruit consumption with immediate recall were independent of those of tofu and, in separate analyses, also of tempeh consumption. On the other hand, weekly consumption of fruit had no significant association. This may be because of low resolution in these data as, of the total cohort, only 15 elderly ate fruit daily, which had previously been found to be a protective factor against dementia (Hogervorst et al., 2008). There may be a further role here for public health messages promoting higher fruit intake.

Limitations of the present study are that we did not include medical examination data in this study and could therefore not control for medication use and morbidity (including dementia), which could have affected results. This could be a reason why no associations were found of food intake in those with an average age of 80 years in this study who may have been more at risk for morbidity (e.g. chronic infectious disease), which could have potentially overruled smaller protective effects of nutrition on cognitive function. However, all participants were community-dwelling and fit enough to travel by car without apparent acute systemic morbidity. Survivor effects, with only the very healthy surviving to an old age, limit generalization of results, and this will be investigated in detail using the follow-up data. Soy products are thought to protect against many age related morbidities, such as cancer and heart disease, which may have also explained positive associations with immediate recall function, rather than through the reported positive effects of isoflavones on brain cells directly (Zhao et al., 2002; Zhao and Brinton, 2007). Other limitations in the present study are the reliance on self report which could confound associations. Those with memory issues would be more likely to underreport soy consumption, similar to data found for hormone treatment (Petitti et al., 2002). However, all carers were present and confirmed answers were given by participants. Where there was discrepancy, the answer of the carer was used. In addition, positive associations were mainly found in those with an average age of 67 years, where recall issues would not be considered to be a major confounding issue, as dementia prevalence starts to increase mainly after 65 years of age.

Data may reflect findings from estrogen treatment where positive effects were most apparent in those who are middle-aged but where no or negative effects were seen in those over 65 years of age (Hogervorst et al., 2009b). Using isoflavone supplements after the menopause has become more popular as an alternative to combat menopausal symptoms (such as hot flashes, night sweats, and cognitive complaints). Short-, but not long-term, treatment studies have shown positive effects of isoflavones on cognitive function (File et al., 2005; Gleason et al., 2009; but see Maki et al., 2009). These data may have a similar pattern to that of estrogen treatment, which probably only has a limited duration positive effect on cognition. Meta-analyses showed positive effects of estrogen treatment on some cognitive tests but only up to 2–3 months (Hogervorst et al., 2002b; Hogervorst et al., 2009b). It may be that different estrogen or soy treatment regimes with intermittent exposure to limit desensitization to continuous treatment could prolong positive effects (Hogervorst, 2009c). Alternatively, genetic subgroups could exist for whom estrogen treatment is not indicated as it increases dementia risk due to formation and accumulation of toxic estrogenic metabolites (Hogervorst et al., 2009a). Long-term, well-controlled, large treatment studies into the effects of soy products on the brain are thus necessary. One relatively recent treatment study showed not only some positive effects of a high-dose 6 month treatment with 100 mg soy isoflavones but also some of placebo in older men and women over 62 years of age (Gleason et al., 2009). In sum, despite not finding significant negative associations in this study, it should be noted that not all that is "natural" is a priori more healthy especially for those over 65 years of age, for whom long-term, high-dose, continuous exposure to estrogenic compounds may not be indicated to preserve brain integrity.

4. Experimental procedures

Rural, community-dwelling elderly of Central Java were included in this survey. Participants had to be over 56 years of age to be included and the villages they resided in had to be covered by the local community health districts of rural Borobudur and Salaam (a 2 h drive from Yogyakarta). Of those included, 54% ($n=115$) could be cognitively assessed for follow-up assessment in April 2009 (of the $n=214$ assessed at baseline in 2006; see Hogervorst et al., 2008). This paper concerns the cross sectional data of the total rolling cohort collected in April 2009, which also included novel participants ($n=33$) who had reached the age of 56 years in 2009. Data on tofu consumption were available for $n=151$. Ethical approval from Loughborough University and the University of Indonesia, governmental permits, and informed

consent were obtained before data collection. Participants had been informed of the research during their visits to the health clinics weeks before testing. They had been made aware that non-participation would in no way affect their treatment at the health center and that this was not obligatory. However, because of the excellent relationship between health center staff and the elderly, the willingness to participate in research was high. Participants were collected by car to travel to the research setting at an agreed date with their carer (only 2 came alone) who also signed the informed consent. This approach had a 96% response rate of those contacted, as only 7 participants of those contacted refused to be tested because of fatigue or frailty on the test day. All others consented to participate. The research was carried out in 1 day in the 5 rooms of the home of one of the administrators of the integrated health unit (Pos Pelayanan Terpadu/Posyandu), which was quiet and which had no distractions. Assessments included cognitive testing and a survey consisting of standardized interview questions, which were carried out by trained researchers.

4.1. *Demographics and lifestyle variables*

The survey consisted of questions about demographics (e.g. age, education, occupation), including an 18-item household goods and luxury questionnaire (electronics, transportation, luxury items) as an indicator of socioeconomic status, and lifestyle variables (e.g. activity, diet, and health). Diet was assessed using a food frequency questionnaire (see Hogervorst et al., 2008; Frankenfeld et al., 2004), which included questions about "regular" consumption of products (yes or no), which were followed by questions on the frequency of daily, weekly, or monthly consumption of these products, such as tempeh and tofu. These questions were all transformed into weekly consumption (e.g. eating tofu once daily was calculated as 7 times a week, eating tofu 3 times a day for 7 days a week was calculated as tofu consumption 21 times a week, whereas once a month was calculated as 0.25 times a week, etc.).

4.2. *Medical examination and cognitive assessments*

A medical examination and a health resume including a carer's report were based on the Cambridge Mental Disorder of the Elderly Examination (CAMDEX; Roth et al., 1988). This also included a psychiatric assessment and a depression scale. Depression was not considered to be severe enough to interfere with cognitive function in any of the participants. None of the participants displayed symptoms of anxiety or other psychiatric morbidity, but one participant with psychosis was excluded. Alcohol was not consumed by any of the participants, all of whom were Muslim.

The modified Hopkins Verbal Learning Test (HVLT; Brandt, 1991; Hogervorst et al., 2008) was administered by trained research assistants. The HVLT is a short (5–10 min) test that is well tolerated. It is a memory test consisting of 12 words which belong to 3 categories (human shelter, precious stones, and animals for list A). The modified test was adapted to local knowledge (e.g. some of the semi precious stones were not known by the local population in a pilot study). It had been translated into Bahasa Indonesia and Javanese and was then back-translated to English by an independent party successfully. Outcomes include the total immediate recall (IR, which assesses learning ability over 3 trials) and the delayed recall (DR), which is done after a 20–30 min interval during which other tests are done or questionnaires are filled in. Memory is one of the earliest functions to decline in dementia (McKhann et al., 1984; American Psychiatric Association, 1994). The total immediate recall of the HVLT had high sensitivity and specificity for dementia when assessed in different cohorts in the US and UK (Brandt, 1991; Hogervorst et al., 2002a; De Jager et al., 2003). Immediate recall of word lists is also sensitive to effects of estrogenic compounds (Hogervorst et al., 2002b) and therefore was the main outcome parameter we reported on in this paper. Test performance distribution in rural Java was normal and was similar to that of a similar aged population in Oxfordshire (Hogervorst et al., 2002a).

4.3. *Statistical analyses*

All data were checked for missing values and potential outliers. Non-parametric Spearman's correlation analyses were performed to assess associations between variables. Linear regression analyses were carried out to investigate whether tofu and tempeh intake was associated with immediate recall function, independent of socioeconomic status (as assessed by household goods) and regular intake of fruits (to control for having an overall healthier dietary pattern) in a first step, and age, education, and gender in a second step. We stratified analyses for age using two different methods. One used a median split, the other investigated the subgroup of middle-aged participants who were younger than 66 years of age (who were considered to be "middle-aged") versus the other group who were older to test the "Window of Opportunity" hypothesis. All analyses were performed in SPSS 17.0 using a p-value of 0.05.

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Abstract: Previous reports have suggested that high frequent tofu consumption is associated with worse cognitive function in East Asian elderly. Some studies also found an increased risk of dementia with high tofu consumption in those older than 65 years of age. Tofu and other soy products, such as tempeh, contain high levels of plant estrogens or isoflavones. This study revisited a rural Central Javanese population (56-97 years of age) who were covered by the Borobudur District Health Centers. Data on cognitive performance were available for n=142 participants. Results showed positive linear associations of weekly tofu (beta= .22, $p<0.05$) and tempeh (beta=.23, $p<0.01$) consumption with immediate recall, which were significant in those with an average age of 67 years. In those with an average age of 80 years, the earlier reported negative association of tofu with immediate recall was no longer significant. Lifestyle changes (reduction of tofu consumption after dissemination of results) or 'healthy survivor effects' may have been responsible for this finding. These findings may be reminiscent of the 'Window of Opportunity' theory, which suggests that estrogenic compounds can exert positive effects on verbal memory, but not in older men and women, when no or negative effects of these compounds on brain cells and cognition have been found. Long-term, placebo-controlled treatment studies should investigate whether tempeh, a fermented soybean product that also contains folate, can maintain cognitive function in middle-aged and elderly participants

***Cover Letter**

Loughborough 12-10-2010

Dear Editor,

Thank you for allowing us to resubmit the paper Borobudur revisited. We would like to thank both reviewers for their useful comments and kind words. We have changed the manuscript according to the reviewer's suggestions as described in more detail in our response to reviewers document

Thank you kindly for this review, again it has further improved our paper

Kind regards

Eef Hogervorst

Loughborough 12-10-2010

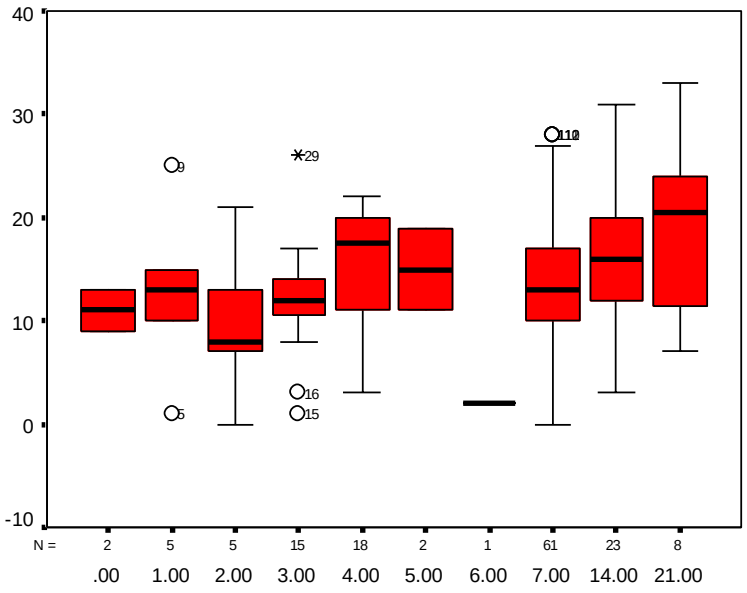
Dear Editor

This is our response to the reviewer's comments

Reviewer's comments:
Regarding the title, my initial problem with the title stemmed from the lack of clarity in terms used to describe the cohorts and the redundancy of "old" and "elderly". Furthermore, terms such as "middle-aged" and "elderly" have specific connotations that may not be applicable to this study.
Taking the reviewer's comments into account, the title was changed to: Borobudur revisited: soy consumption may be associated with better recall in younger, but not in older, rural Indonesian elderly

Results, Discussion and Methods:
Figure 1 is not adequately labeled, especially for a non-specialist in the field. Although the figure legend describes the axes, there is no y-axis label, nor are there units. On the x-axis "tempe" should be capitalized and spelled correctly, and there should be some indication that "Tempeh per week" refers to consumption. Units should be indicated

Fig 1. Box plots (with the bottom and top of the box being the 25th and 75th percentile (the lower and upper quartiles, respectively), and the band near the middle being the median, with outliers) of the total immediate recall performance on the HVLT ('tot recall', in number of words recalled over 3 trials, with a maximum of 36 words recalled in total) and the frequency of tempeh consumed per week on the y-axis, with n referring to numbers of participants per boxplot.



The frequency of tempeh consumed per week (e.g. 21 is 3 times per day, '7' is daily, '2' is twice a week etc.)

1. In the last sentence of the Abstract, last sentence should read, "Long-term, controlled-treatment studies.soybean product that also contains.".
We have changed this as suggested

2. On page 5, the abbreviation for television must be capitalized (TV).
We have changed this as suggested

3. On page 6, 3rd paragraph, "well educated" should be hyphenated (well-educated). Same paragraph, "Better educated" should also be hyphenated.
We have changed this as suggested

4. On page 9 in the Discussion, I assume that "Jakarta Post" is a publication, and the "p" should be capitalized if it is a title. This might be due to citation software issues. Later in that paragraph, the sentence that begins, "It is also unclear." the parentheses and question mark surrounding "still" should be removed so that it reads: ".whether laced tofu is still sold.".
We have changed this as suggested

5. On page 11, 2nd paragraph, the sentence beginning, "Short (but not longer) term." as constructed is visually and grammatically awkward. I suggest eliminating the parentheses, such that it reads, "Short-, but not long-term, treatment studies have shown.".
We have changed this as suggested

6. On page 11, 2nd paragraph, line 16, the sentence should read, "Long-term, well-controlled, large treatment studies.".
We have changed this as suggested

7. On page 11, 2nd paragraph, line 22, the sentence should read, "whom long-term, high-dose, continuous exposure.".
We have changed this as suggested

8. In the first sentence of page 12, the sentence should read, "Rural, community-dwelling elderly."
We have changed this as suggested

Thank you kindly for this review, again it has further improved our paper

Kind regards

Eef Hogervorst

To submit your revision, please upload your revised files, including a point-by-point response to the reviewers' comments, to the Elsevier Editorial System (http://ees.elsevier.com/bres/).

Borobudur revisited: soy consumption may be associated with better recall in younger, but not in older, rural Indonesian elderly

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Abstract

Previous reports have suggested that high frequent tofu consumption is associated with worse cognitive function in East Asian elderly. Some studies also found an increased risk of dementia with high tofu consumption in those older than 65 years of age. Tofu and other soy products, such as tempeh, contain high levels of plant estrogens or isoflavones. This study revisited a rural Central Javanese population (56-97 years of age) who were covered by the Borobudur District Health Centers. Data on cognitive performance were available for n=142 participants. Results showed positive linear associations of weekly tofu (beta= .22, $p<0.05$) and tempeh (beta=.23, $p<0.01$) consumption with immediate recall, which were significant in those with an average age of 67 years. In those with an average age of 80 years, the earlier reported negative association of tofu with immediate recall was no longer significant. Lifestyle changes (reduction of tofu consumption after dissemination of results) or 'healthy survivor effects' may have been responsible for this finding. These findings may be reminiscent of the 'Window of Opportunity' theory, which suggests that estrogenic compounds can exert positive effects on verbal memory, but not in older men and women, when no or negative effects of these compounds on brain cells and cognition have been found. Long-term, placebo-controlled treatment studies should investigate whether tempeh, a fermented soybean product that also contains folate, can maintain cognitive function in middle-aged and elderly participants (231 words).

List of abbreviations

AD=Alzheimer's disease
E2=estradiol
DR=delayed recall
IR=immediate recall
HT=hormone therapy
HVLT= Hopkins Verbal Learning Test
MMSE=Mini Mental Status Examination
SD=standard deviation
SES=socioeconomic status
WHIMS =Women's Health Initiative Memory Study

Disclosure

We have nothing to disclose and none of the authors has any vested interest in any of the products mentioned in this paper. All authors have contributed to the paper.

Acknowledgements

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We are grateful to all test-assistants and students who helped us collect the data and all the participants and staff of health centers. Without them this paper would not have been written.

Introduction

Previous studies investigating the potential of plant estrogens, such as isoflavones, to protect the aging brain and cognitive function have found contradicting results (see (Zhao and Brinton 2007; Clifford, Yesufu et al. 2009; Hogervorst, Yesufu et al. 2009; Yesufu, Rahardjo et al. in press), for reviews). Because of their chemical similarity to estrogens, isoflavones can exert estrogenic effects on the brain, particularly when endogenous estrogen levels are low (Zhao and Brinton, 2007; (Yesufu, Rahardjo et al. in press). Soy based products, such as tofu and tempe, contain high levels of isoflavones (Wang and Murphy 1994). Although some observational studies have not found an association between cognition and isoflavone intake in persons with low soy consumption (Yesufu, Rahardjo et al. in press), high regular consumption of the isoflavone rich tofu paradoxically increased dementia risk in older (> 65 years of age) East Asian populations (Rice, Graves et al. 2000; White, Petrovitch et al. 2000; Hogervorst, Sadjimin et al. 2008). In those older than 68 years of age, we found negative linear associations of the potent isoflavone genistein with cognitive function (Hogervorst, Yesufu et al. 2009).

However, we also found curvilinear associations of genistein with better immediate recall performance in middle-aged (< 68 years of age) men and women who were closer to the age of menopause. This suggests that optimal levels of genistein were associated with better memory function in the middle-aged (Hogervorst, Yesufu et al. 2009). Similarly, randomised controlled treatment studies mainly found positive effects of soy based isoflavones on cognition in women close to the age of menopause, with no or mixed effects in the older population (Yesufu, Rahardjo et al. in press). These findings may be reminiscent of the 'Window of Opportunity' theory, which is supported by animal and observational data and suggests that effects of estrogens are most prominent when treatment is given close to the age of menopause, when organ systems are still responsive to estrogens' effect (Henderson, Benke et al. 2005).

However, this theory does not explain negative effects of estrogens and estrogenic compounds in older participants. Using assays of sufficient sensitivity, and controlling for morbidity and medication use affecting sex steroid levels, several investigators reported that women with Alzheimer's disease (AD), the most common type of dementia in the West (Zhao and Brinton 2007), actually had slightly higher levels of estrogens than controls (Cunningham, Sinnott et al. 2001; Hogervorst, Williams et al. 2003; Paoletti, Congia et al. 2004). Longitudinal studies also confirmed increased risk for dementia with higher levels of estrogen in elderly women (Geerlings, Strozyk et al. 2006);(Ravaglia, Forti et al. 2007). In

addition, high levels of estrogens in elderly women and men (> age 65 years) are not necessarily associated with better brain function (Yaffe, Lui et al. 2002; Den Heijer, Geerlings et al. 2003; Muller, van den Beld et al. 2009; Hogervorst, Matthews et al. 2010). These data may also be in accordance with data of the Women Health Initiative Memory Study (WHIMS) which reported negative effects of estrogen treatment on cognitive function (Espeland, Rapp et al. 2004) and an increased risk of dementia in women over 65 years of age (Shumaker, Legault et al. 2003; Shumaker, Legault et al. 2004).

These negative associations of estrogens and estrogenic compounds (such as genistein, which is abundant in tofu) with brain function in older (> 65 years of age) participants may be explained by the 'Healthy cell bias' hypothesis (Brinton 2008; Brinton 2008). This hypothesis is based on cell culture studies which show that brain cells, which are undergoing pathological change (which is more likely to be the case in older neurons) do not actually benefit from an estrogenic environment and are more likely to show accelerated demise in this environment. This is different for healthy neurons, which show benefit from an estrogenic environment. This hypothesis both explains positive effects of estrogens on the brains of younger, middle-aged women and younger (but not older) ovarietomized animals (Gibbs 2009), but also the negative effects of estrogens and estrogenic compounds on cognition in older women and men.

A caveat is that we also found positive effects of tempeh in the Indonesian study, when it was entered in the same statistical model, and where it seemed to off-set the negative association of high tofu intake with memory (Hogervorst, Sadjimin et al. 2008). Tempeh is a fermented whole soybean product which is usually found to contain higher levels of genistein and other isoflavones than tofu (Wang and Murphy 1994). In addition, due to fermentation with molds, levels of folate are also increased in this food product (Ginting and Arcot 2004). In our earlier analyses of the Oxford Project To Investigate Memory and Ageing data, we found that older women with higher levels of estrogens had a higher risk of dementia (Hogervorst, Williams et al. 2003). However, when women also had high levels of serum folate, they did not perform under the cut-off scores for dementia on the Mini-Mental Status Examination (MMSE) (Hogervorst and Smith 2002). It may thus be the case that folate or other compounds in tempeh protect against potentially negative effects of estrogenic compounds in the elderly.

We revisted rural Central Java where we previously found the strongest negative associations of tofu consumption with immediate recall in those over 68 years of age (Hogervorst, Sadjimin et al. 2008) to further investigate the association of different soy

products with memory function. On the basis of the previous studies done, we hypothesized that tempeh (because it contains folate) would show protective associations with immediate recall in both the middle-aged (65 years of age and younger) and those who were 66 years of age and older. In separate analyses tofu was expected to only show this positive association in the middle-aged, but to have a negative association with immediate recall in those older than 65 years. Immediate recall was previously shown sensitive to dementia (Hogervorst et al, 2002B), estrogens (Hogervorst et al., 2002C) and soy product consumption (Hogervorst et al, 2008). We also performed a more in depth socioeconomic assessment to investigate whether this mediated the association of soy products with cognition. People of low socioeconomic status were hypothesized to eat more soy (as an animal protein substitute, because it is cheaper). However, low socioeconomic status in itself is associated with an faster cognitive decline and an earlier onset of dementia (Karp, Kareholt et al. 2004) and could thus have mediated the association between high soy intake and low memory function.

Results

Cognitive data were available of n=142 rural participants. Almost half had not attended school, and most others had only obtained primary schooling. Many (45%) still worked as farmers or labourers and people on average only had 5 household goods or luxuries indicating socioeconomic status, of which the most frequent were: electricity (98%), a TV (71%), a radio (57%), livestock (56%), and a bicycle (49%). Socioeconomic status (SES) as assessed by these parameters would on average thus be low for the villagers, but data distribution was not skewed, showing a good spread in goods available to villagers.

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Median and mean tempeh and tofu consumption was daily (i.e. 7 times a week, SD=5), ranging from never to three times a day. In comparison, only 18% of this sample ate fruit 'regularly' and of these participants only half did so on a daily basis (n=15). The average weekly consumption of fruit was only 2 pieces of fruit (SD=2.5), despite availability in the area.

Spearman's rank correlations suggested that higher weekly tempeh consumption was associated with better immediate recall, as measured using the 3 trials of the Hopkins Verbal

Learning Test (HVLT IR, rho=.19, p<0.05), with a similar trend for tofu (rho=.16, p=0.06, see figure 1). Regular (but not weekly) fruit consumption was also associated with better immediate recall (HVLT IR, rho=.18, p<0.05). Delayed recall as measured by using the HVLT was not associated with intake of any of the foods (p>0.28).

Other factors associated with food intake

Fruit intake, when calculated in consumption per week, was only positively associated with total tempeh and tofu intake per week (rho=.30, =.41, p<0.001). Those who ate more tempeh also ate more tofu (rho=.82, p<0.001). This suggests dietary patterns (healthy patterns or perhaps patterns reflecting abundance). Unfortunately, we did not have data on overall caloric intake to calculate the proportionate intake of these healthy foods.

Tempeh and tofu consumption was higher in more educated people (rho=.24 and .19, p<0.05 resp) and (in contrast to our expectations), tofu was also more likely to be consumed by those of higher socioeconomic status (SES), as assessed by number of household goods or luxuries (rho=.18, p<0.05). Box plots showed that those who were farmers or laborers had a maximum daily median intake of tofu, but also that more than half of the entrepeneurs were able to consume more than that and that these ate tofu 2 or 3 times a day. A similar trend (p=0.07) existed for tempeh with SES, but this did not exist for fruit consumption. Older people were in general less well-educated (rho=-.28, p<0.001) and had fewer household goods or luxuries (rho=-.29, p<0.001). Better-educated people also had more household goods (rho=.36, p<0.0001).

Other factors associated with immediate recall

Immediate recall was associated with having had more education (rho=.41, p<0.001) and having more household goods (rho=.18, p<0.05). Those who were older had worse immediate recall (HVLT IR rho=-.39, p<0.001). There was no gender difference on immediate recall performance, perhaps because there was no age difference between men and women of this cohort, or because women had received on average less education (p<0.001) than men.

Using linear regression indicated that there were significant associations of tofu (beta=.22, $p<0.05$) and tempe[1] (beta=.23, $p<0.01$, see fig 1) with immediate recall performance (HVLT IR total recall, but not DR), which was independent of SES and weekly fruit intake, which were both non-significant. However, associations between soy consumption and immediate recall were no longer signficant when age, sex and education were also entered in the model. A trend remained for weekly tofu consumption (beta=.14, p=.10) to be associated with better immediate recall, when the other covariates were entered (age: beta=-.29, $p<0.001$, education, beta=.33, $p<0.0001$ gender, p=.78, SES, p=.97). In this model, regular fruit consumption (beta=.15, $p<0.05$) was also independently associated with better immediate recall.

Delayed recall showed no associations with soy product consumption or fruit intake using similar analyses

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In analyses stratified for median age, in those who were younger than 73 years of age (n=74, mean age 67 years), weekly tofu consumption was a significant predictor of immediate recall (beta=.21, p=.05, and a similar trend existed for tempe: beta=.19, p=0.09). This association remained significant after addition of the covariates. Of these covariates, only education (beta=.47, $p<0.001$) was an independent predictor of better performance. Age, SES and gender were non significant contributors to this model. There was also a trend for regular (but not weekly) fruit consumption (beta=.18, p=.09) to be associated with better performance in those younger than 73 years.

However, these associations were not replicated in those who were older than 73 years of age (n=68, mean age 80 years), where age was the only significant predictor of low immediate recall (beta=-.40, p=0.006). However, in this group, the direction of the association between tofu and immediate recall was negative (but this did not reach significance).

Using a different stratification by investigating those participants who were younger than 66 years of age (which included 20% of the sample, and who were considered to be 'middle-aged' to investigate the 'Window of Opportunity' hypothesis) versus those who were older, tempeh (beta=.47, $p<0.05$) and tofu (beta=.49, $p<0.05$) both had a significant association with immediate recall in unadjusted models, but again only in those younger than 66 years of age,

[1] Tempeh and tofu were entered separately, as there was significant co-linearity

and not in the older participants or when analyses were adjusted for covariates. Entering curvilinear terms did not not result in better fits within the models, indicating no optimal levels of soy consumption for optimal immediate recall. Delayed recall again showed no associations with soy product consumption using similar analyses.

Discussion

Results reflect earlier findings of regular consumption of soy products and fruit having positive associations with immediate recall in those with an average age of 67 years of age (< 73 years of age), but not in those with an average age of 80 years of age. In this study, we further investigated whether low socioeconomic status mediated the negative associations found in earlier studies between frequent tofu consumption and cognition. However, the opposite was found, with rural elderly who had more household goods and luxuries as indicators of socioeconomic status eating more soy products than those who had less. Socioeconomic status also did not mediate the associations found.

Delayed recall, which is considered by some to be a more sensitive measure of impeding dementia, was in the present and earlier studies not associated with soy consumption. With an average of 5 words recalled, resolution was low for these analyses. In our earlier work, the total immediate recall showed similar sensitivity and specificity for dementia as the delayed recall (Hogervorst, Combrinck et al. 2002) but immediate recall has a wider spread of data, creating better resolution for data analyses. Similar test sensitivity issues were also seen for estrogenic compounds, where not all verbal memory tests are equally affected (Sherwin 1994); and meta-analyses indicated that immediate recall of word lists seemed most sensitive to effects of estrogen treatment than delayed recall (Hogervorst, Yaffe et al. 2002).

This study thus extends and confirms our earlier work suggesting that tempeh had protective associations with immediate recall, although the earlier reported negative associations of high tofu consumption with immediate recall were not significant in the present study. This was possibly because of a smaller sample size in this cohort as compared to that the previous study (n=214) (Hogervorst, Sadjimin et al. 2008). Alternatively, lifestyle changes may be responsible for this findings, as all participants had been briefed about the negative associations of tofu and memory found after data analyses. Post hoc analyses showed that, of those investigated and followed up in this paper, only 21% ate tofu more than once a day, whereas at baseline testing this was almost double (38%), suggesting that more

people had cut down on tofu consumption (or alternatively, that more of those with high consumption had died earlier). In 2009, more people ate tofu just once a day (48%) compared to 30% at baseline in 2007.

Another hypothesis from our earlier work (Hogervorst, Sadjimin et al. 2008) was that negative associations of tofu with immediate recall and dementia risk in those over 68 years (in that paper) were found because of addition of formaldehyde to tofu to preserve its freshness. Several factories in Jakarta had apparently sold tofu laced with this highly neurotoxic compound to traditional markets (see Jakarta Post 07/31/2009 and 09/11/2008) and according to the Department of Public Health this practice was widespread. However, when we tested for this in 2009 in various tofu samples of shops and vendours around Jakarta, we could not find evidence of addition of formaldehyde to tofu (Aryani, 2009, PhD thesis Universitas Indonesia). It is also unclear whether laced tofu is still sold in rural Central Java and more research needs to be done to investigate this association. This practice does not occur in tempeh, which can be kept fresh for longer due to fermentation of this product.

Using curvilinear terms, we did not find optimal levels of soy consumption in those with an average age of 67 years, although in this age group we earlier had reported optimal levels of salivary genistein, the most active estrogenic compound (Hogervorst et al., 2009A). The Study of Women's Health Across the Nation (SWAN) did not find optimal calculated levels of genistein and daidizein in their East Asian middle-aged women (Huang, Luetters. C. et al. 2006). However, in this study isoflavone levels were inferred from Food Frequency Questionnaires, and so these were not measured directly, similar to the present study where we only included Food Frequency data. The authors also used tertiles to investigate optimal genistein and daidizein levels, rather than using continuous variables as we did earlier. The SWAN also did not report *weekly* soy intake as was done in the other and present studies reporting significant associations with cognitive function. For instance, in the Honolulu Aging Asian Study, Japanese-American men and women who consumed tofu more than twice a week in midlife and in old age had a higher risk for dementia, lower brain weight and more ventricular enlargement (White, Petrovitch et al. 2000). In the Kame project (Rice, Graves et al. 2000), Japanese-American women over age 65 years who consumed tofu more than three days a week had lower cognitive performance. In stratified analyses, this remained significant only for those women taking hormone therapy, suggesting a cumulative negative effect of estrogenic compounds on cognition. In the present Javanese cohort studied, none of the women took hormone therapy, but 5 reported to have not undergone menopause yet. However, post hoc analyses of soy product consumption and its association with immediate

recall performance excluding these participants were not substantially different from those currently presented in the results section. Lastly, in the Kame project (Rice, Graves et al. 2000), at 2 year follow-up, all those who had moderate tofu consumption had most cognitive improvement over time. Where optimal soy intake lies at what age for maintenance of cognitive performance over time and how this is associated with other dietary intakes and possibly genetic profiles relating to sex steroid metabolism and synthesis (Hogervorst and Bandelow 2007) remains to be further investigated. Other studies including elderly women with Western diets with low average soy intake have not found any associations with cognition (Yesufu, Rahardjo et al. in press), which may further suggest that dosage and age play an important role rather than consumption per se.

As there were also positive associations of both soy products with higher fruit consumption, healthy dietary patterns may have confounded the positive associations reported in the present study. However, positive associations of regular fruit consumption with immediate recall were independent of those of tofu, and in separate analyses, also of tempeh consumption. On the other hand, weekly consumption of fruit had no significant association. This may be because of low resolution in these data, as of the total cohort only 15 elderly ate fruit daily, which had previously been found to be a protective factor against dementia (Hogervorst, Sadjimin et al. 2008). There may be a further role here for public health messages promoting higher fruit intake.

Limitations of the present study are that we did not include medical examination data in this study and could therefore not control for medication use and morbidity (including dementia), which could have affected results. This could be a reason why no associations were found of food intake in those with an average age of 80 years in this study who may have been more at risk for morbidity (e.g. chronic infectious disease), which could have potentially overruled smaller protective effects of nutrition on cognitive function. However, all participants were community dwelling and fit enough to travel by car without apparent acute systemic morbidity. Survivor effects, with only the very healthy surviving to an old age, limit generalisation of results and this will be investigated in detail using the follow-up data. Soy products are thought to protect against many age related morbidities, such as cancer and heart disease, which may have also explained positive associations with immediate recall function, rather than through the reported positive effects of isoflavones on brain cells directly (Zhao, Chen et al. 2002; Zhao and Brinton 2007). Other limitations in the present

study are the reliance on self report which could confound associations. Those with memory issues would be more likely to underreport soy consumption, similar to data found for hormone treatment (Petitti, Buckwalter et al. 2002). However, all carers were present and confirmed answers given by participants. Where there was discrepancy, the answer of the carer was used. In addition, positive associations were mainly found in those with an average age of 67 years, where recall issues would not be considered to be a major confounding issue, as dementia prevalence starts to increase mainly after age 65 years.

Data may reflect findings from estrogen treatment where positive effects were most apparent in those who are middle-aged, but where no or negative effects were seen in those over 65 years of age (Hogervorst, Bandelow, 2009B). Using isoflavone supplements after the menopause has become more popular as an alternative to combat menopausal symptoms (such as hot flashes, night sweats and cognitive complaints). Short-, but not long-term, treatment studies have shown positive effects of isoflavones on cognitive function (File, Hartley et al. 2005; Gleason, Carlsson et al. 2009); but see (Maki, Rubin et al. 2009). These data may have a similar pattern to that of estrogen treatment, which probably only has a limited duration positive effect on cognition. Meta-analyses showed positive effects of estrogen treatment on some cognitive tests, but only up to 2 to 3 months (Hogervorst, Yaffe et al. 2002; Hogervorst, Yaffe et al. 2009). It may be that different estrogen or soy treatment regimes with intermittent exposure to limit desensitisation to continuous treatment could prolong positive effects (Hogervorst, Yaffe et al. 2002C). Alternatively, genetic subgroups could exist for whom estrogen treatment is not indicated as it increases dementia risk due to formation and accumulation of toxic estrogenic metabolites (Hogervorst, Bandelow et al. 2009). Long-term, well-controlled, large treatment studies into the effects of soy products on the brain are thus necessary. One relatively recent treatment study showed some positive effects of a high dose 6 month treatment with 100 mg soy isoflavones, but also some of placebo in older men and women over 62 years of age (Gleason, Carlsson et al. 2009). In sum, despite not finding significant negative associations in this study, it should be noted that not all that is ‘natural’ is a priori more healthy, especially for those over 65 years of age, for whom long-term, high-dose, continuous exposure to estrogenic compounds may not be indicated to preserve brain integrity.

Methods

Rural, community-dwelling elderly of Central Java were included in this survey. Participants had to be over 56 years of age to be included and the villages they resided in had to be covered by the local community health districts of rural Borobudur and Salaam (a 2 hour drive from Yogyakarta). 54% (n=115) of those included could be cognitively assessed for follow-up assessment in April 2009 (of n=214 assessed at baseline in 2006, see Hogervorst, 2008). This paper concerns the cross sectional data of the total rolling cohort collected in April 2009, which also included novel participants (n=33) who had reached the age of 56 years in 2009. Data on tofu consumption were available for n=151. Ethical approval from Loughborough University and the University of Indonesia, governmental permits and informed consent were obtained before data collection. Participants had been informed of the research during their visits to the health clinics in the weeks before testing. They had been made aware that non participation would in no way affect their treatment at the health center and that this was not obligatory. However, because of the excellent relationship between health center staff and the elderly, the willingness to participate in research was high. Participants were collected by car to travel to the research setting at an agreed date with their carer (only 2 came alone) who also signed the informed consent. This approach had a 96% response rate of those contacted, as only 7 participants of those contacted refused to be tested because of fatigue or frailty on the test day. All others consented to participate. The research was carried out in one day in 5 rooms of the home of one of the administrators of the integrated health unit (Pos Pelayanan Terpadu/Posyandu), which was quiet and which had no distractions. Assessments included cognitive testing and a survey consisting of standardized interview questions, which were carried out by trained researchers.

Demographics and lifestyle variables

The survey consisted of questions about demographics (e.g. age, education, occupation), including an 18 item household goods and luxury questionnaire (electronics, transportation, luxury items), as an indicator of socioeconomic status; and lifestyle variables (e.g. activity, diet and health). Diet was assessed using a Food Frequency Questionnaire (see Hogervorst, Sadjim et al, 2008; Frankenfeld, 2004), which included questions about 'regular' consumption of products (yes or no), which were followed by questions on the frequency of daily, weekly, or monthly consumption of these products, such as tempeh and tofu. These questions were all transformed into weekly consumption (e.g. eating tofu once daily was calculated as 7 times a week, eating tofu 3 times a day for 7 days a week was calculated as

tofu consumption 21 times a week, whereas once a month was calculated as 0.25 times a week etc.).

Medical examination and cognitive assessments

A medical examination and a health resume including a carers report were based on the Cambridge Mental Disorder of the Elderly Examination (CAMDEX, (Roth, Huppert et al. 1988). This also included a psychiatric assessment and a depression scale. Depression was not considered to be severe enough to interfere with cognitive function in any of the participants. None of the participants displayed symptoms of anxiety or other psychiatric morbidity, but one participant with psychosis was excluded. Alcohol was not consumed by any of the participants, all of whom were Muslim.

The modified Hopkins Verbal Learning Test (HVLT, Brandt, 1991; Hogervorst, Sadjim, 2008) was administered by trained research assistants. The HVLT is a short (5-10 min) test that is well tolerated. It is a memory test consisting of 12 words which belong to 3 categories (human shelter, precious stones and animals for list A). The modified test was adapted to local knowledge (e.g. some of the semi precious stones were not known by the local population in a pilot study). It had been translated into Bahasa Indonesia and Javanese and was then back translated to English by an independent party successfully. Outcomes include the total Immediate Recall (IR, which assesses learning ability over 3 trials) and the Delayed Recall (DR), which is done after a 20-30 min interval during which other tests are done or questionnaires are filled in. Memory is one of the earliest functions to decline in dementia (McKhann, Drachmann et al. 1984; Association 1994). The total immediate recall of the HVLT had high sensitivity and specificity for dementia when assessed in different cohorts in the US and UK (Brandt, 1991; (Hogervorst, Combrinck et al. 2002; De Jager, Hogervorst et al. 2003). Immediate recall of word lists is also sensitive to effects of estrogenic compounds (Hogervorst, Yaffe et al. 2002) and therefore was the main outcome parameter we reported on in this paper. Test performance distribution in rural Java was normal and was similar to that of a similar aged population in Oxfordshire (Hogervorst, Combrinck et al. 2002).

Statistical analyses

All data were checked for missing values and potential outliers. Non-parametric Spearman's correlation analyses were performed to assess associations between variables. Linear regression analyses was carried out to investigate whether tofu and tempeh intake was

associated with immediate recall function, independent of socioeconomic status (as assessed by household goods) and regular intake of fruits (to control for having an overall healthier dietary pattern) in a first step, and age, education and gender in a second step. We stratified analyses for age using two different methods. One used a median split, the other investigated the subgroup of middle-aged participants who were younger than 66 years of age (who were considered to be 'middle-aged') versus the other group who were older to test the 'Window of Opportunity' hypothesis. All analyses were performed in SPSS 17.0 using a p-value of 0.05.

Table 1. Demographic characteristics of rural Central Javanese participants

Variable	percentage or mean (SD)	range
Sex (% Women)	61%	
Ethnicity (Javanese)	100%	
Religion (Muslim)	100%	
Age (mean and median)	73 (SD=8)	56-97
Household goods (#, SES)	4.6 (SD=2.7)	0-15 of 18
Occupation		
Not working/retired	38%	
Famers	30.3%	
Laborers	14.8%	
Business/entrepreneur	12%	
Other	5%	
Education		
No education	48%	
Part of primary school	25%	
Primary School finished	14%	
Secondary School	8.5%	
High School	3.5%	
HVLT total immediate recall	14 word (SD=7.12)	0- 33
delayed recall (max 12)	5 words (SD=2.92).	0- 12

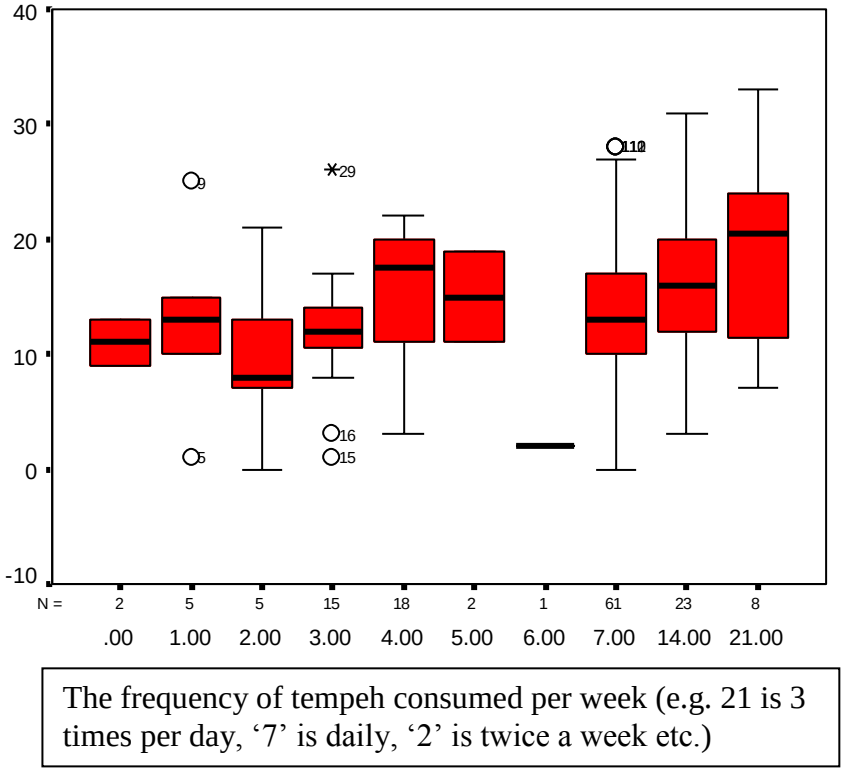

Fig 1. Box plots (with the bottom and top of the box being the 25th and 75th percentile (the lower and upper quartiles, respectively), and the band near the middle being the median, with outliers) of the total immediate recall performance on the HVLT ('tot recall', in number of words recalled over 3 trials, with a maximum of 36 words recalled in total) and the frequency of tempeh consumed per week on the y-axis, with n referring to numbers of participants per boxplot.

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